AN INVESTIGATION INTO AN INNOVATIVE LAND APPLICATION STRATEGY FOR THE SUSTAINABLE MANAGEMENT OF BIOSOLIDS

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DECLARATION

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; and, any editorial work, paid or unpaid, carried out by a third party is acknowledged.

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ABSTRACT

This thesis investigates the use of nutrients from biosolids to produce two valuable crops (canola and oats) as an alterative biosolids management strategy which can be used for energy production and livestock fodder and hence avoids the problem of potential food contamination.

A two year field experiment was conducted at Western Water at Surbiton Park (WWSP) in 2006 and 2007 to compare the responses of soil and plants to the applications of anaerobically digested dewatered biosolids and composted biosolids in canola (*Brassica napus* L.) and oat (*Avena sativa*) under a crop rotation regime in a clay loam soil.

Dewatered biosolids and composted biosolids application rates were calculated based on the nitrogen limited biosolids application rates (NLBAR) for canola and oats and incorporated into the top10 cm of the soil depth on dry weight basis. In 2007, biosolids were reapplied at the same rates as in 2006, but the crops were rotated.

Results of the first year field experiment showed that the optimum canola seed yields were recorded at 25 t ds/ha and 30 t ds/ha of dewatered biosolids and composted biosolids application rates, respectively ($p < 0.05$) whereas, for the oat crop, the optimum seed yields were observed at the 25 t ds/ha and 50 t ds/ha for dewatered biosolids and composted biosolids, respectively ($p < 0.001$ and $p < 0.05$).

In 2007 the optimum canola seed yields were recorded at 45 t ds/ha and 50 t ds/ha dewatered biosolids and composted biosolids rates respectively ($p < 0.001$ and $p < 0.01$). In the second year of the trial the oat crop was infested with stem rust which negatively impacted upon the grain yield. At the optimum biosolids application rates the yields were significantly higher than those obtained from the fertilized control plots for both crops, with the dewatered biosolids treated canola and oats plots producing significantly greater yields than composted biosolids treated plots.

Total N, P and S in dewatered biosolids and composted biosolids amended soils were significantly higher ($p < 0.05$) than the conventionally fertilized and untreated control plots and increased following high biosolids loading rates.

The levels of total N, P and S in canola seed oil increased following biosolids applications. Despite increases in the quantity of oil in canola seed following biosolids applications, seed oil concentration was negatively correlated with concentrations of total nitrogen (TN) in canola seed.

Even though the addition of both biosolids type expected to produce slightly higher oil yields, more N loading had negatively impacted the quality of canola seed oil, dewatered biosolids

rates at 5 t/ha and composted biosolids rates at 50 t/ha produced the highest canola seed oil content (45.2 and 47.5 %), respectively, and thus these application rates would be ideal for farmers who need to maximize the oil content to benefit from the bonification scheme.

A significant increase in total and DTPA extractable heavy metals in biosolids amended soil were also noted showing an upward trend due to both biosolids applications.

The DTPA extractable concentrations of Zn and Fe observed in the canola-oats cropping sequence were higher than the corresponding levels found in the oats-canola rotation.

In the canola-oats cropping sequence considerably higher concentrations of soil total N and Olsen-P and DTPA extractable (Cu, Zn, Fe) were observed than in the oats-canola cropping sequence. It is expected that this would be due to the increased up take of nutrients and metals by canola compared to oats in the first year of the experiment, reducing the N, Olsen-P and DTPA extractable (Cu , Zn, Fe) concentrations in the canola plots. This might have led to higher concentrations of N and P and metals residues to be left in the oats plots in the second year of the experiment in the canola-oats rotation than in the oats-canola cropping sequence.

The highest levels of total Cu and Zn recorded at the 65 and 70 t/ha dewatered biosolids and composted biosolids treated plots did not exceed the maximum EPA Victoria ceiling limits for Cu and Zn (300 and 250 µg/g) , for soils receiving biosolids for crop production (EPA Vic, 2004).

If biosolids are applied at the recommended agronomic rate (1NLBAR for canola which is equivalent to 10 t/ha ds), dewatered biosolids may possibly be applied repeatedly for 64 years before reaching the Victorian EPA maximum ceilling limits for Zn. Using the same logic for the oat crop wherea 1NLBAR is equivalent to 11 t ds/ha , dewatered biosolids it would take 45 successive years. In practice, biosolids would be applied once in a crop rotation (for reasons of P management), therefore in reality it may take >200 years in total to reach the soil limits. Furthermore, the metal accumulation models are based on a cultivation depth of 10 cm, in practice the plough depth may be 25 cm, so the maximum concentration might not be attained for much longer.

From the findings of this study, it is clear that the two biosolids types had different physical properties and nutrient compositions and behaviours, and thus the relevance and assumption of using NLBAR (nitrogen limited biosolids application rate) will not be valid for all types of biosolids products, as nutrient release properties of the two biosolids could be different and possibly depends on other environmental factors including temperature, moisture, crop and soil types. Therefore, it is suggested that biosolids application should take into account the soil and biosolids type and the specific site characteristics of the area receiving the biosolids.

Biosolids applications significantly affected the soil pH, with dewatered biosolids decreasing and composted biosolids increasing the soil pH. The decrease in soil pH due to dewatered biosolids application was correlated with a significantly increased DTPA extractable metal concentration and this was positively correlated with metal uptake by both crops.

The HNO_3/H_2O_2 extractable heavy metals in canola and oats leaves also showed a significant increase due to biosolids applications. The uptake of Cu and Zn by canola crop was substantially higher than the corresponding concentrations observed in oats leaves. Significant positive correlations between DTPA extractable metals and their respective concentrations in plant tissue were also observed, suggesting that DTPA is a reliable estimator of plant available heavy metals in biosolids amended soil.

Higher total N and extractable P concentrations were also observed in canola shoots than in oats treated with dewatered biosolids in both years of the trial suggesting that canola extracted more N and P which was consistent with the higher biomass yield than oats; however, crops did not show any signs of toxicity or deficiency symptoms for N.

Concentration of *E.coli* in dewatered biosolids and composted biosolids treated plots were not statistically different from the control plots and *Salmonella* species in biosolids amended soil were not detected. However, the concentrations of *Clostridium perfringens* varied slightly across the different dewatered biosolids treated plots showing no consistent trend in any of the plots receiving dewatered biosolids.

The results of economic valuation of biosolids showed that the productivity method estimated a higher value (Aus $ 32.00) for one tonne of dewatered biosolids than the nutrient (N, P, K) replacement approach (Aus $ 20.54); conversely, there was no significant difference between the two techniques in estimating the value of one tonne of composted biosolids. The energy value of total canola oil produced per hectare of land using the maximum (54 t/ha) dewatered biosolids application was estimated to be 59,670 MJ/ha which was significantly higher than the corresponding energy value (31,824 MJ/ha) of the canola oil produced using the maximum (94 t/ha) composted biosolids application.

To fully utilize, the 957 tonnes ds anaerobically digested dewatered biosolids generated annually at WWSP environmentally and economically sustainable way at the 5 t/ha to 25 t/ha ds per year for optimum canola seed oil yield production, for this, Wester Water (Surbiton Park) needs annually 38 to 191 ha of land.

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ABBREVIATIONS

ABARE: Australian Bureau of Agricultural and Resource Economics

ABN: Available biosolids nutrient

ASPAC: Australian Soil and Plant Analysis Council

AS 4454: Australian Standards for Composts, soil conditioners and mulches

BDL: Below detection limits

C2/T3: Contaminant grade 2 and treatment grade 3

CFU: Colony forming units

CNR: Crop nutrient requirement

CRM: Certified reference material

ds Dry solids

DM: dry matter

DW: Dry weight basis

DTPA: Diethylene triamine penta acetic acid

EC: Electrical conductivity

EILs: Ecological investigation levels as described in the National Environment Protection Measure (Assessment of Site Contamination) 1999.

FIA: Flow injection analyser

FW: Fresh weight basis

HIL: Health investigation limit

Kt: Kilo tonne

Kg/hL: Kilograms per hector litre

LSD: Least significant difference

NBRP: National Biosolids Research Program

NEPC: National Environment Protection Council

NEPM: National Environment Protection and Management

NLBAR: nitrogen limited biosolids application rates

NIST: National Institute for Standards and Technology

PFU: Plaque forming units

SRM: Standard reference material

STD: Standard Reference Material

WD-XRF: Wave length dispersive X- ray fluorescence spectrometry

WWSP: Western Water at Surbiton Park

WWT: Waste Water Treatment

DEFINITIONS

Biosolids: Organic solids derived from sewage treatment processes that are in a state that they can be managed to sustainably utilize their nutrient, soil conditioning, energy, or other value (i.e. achieve minimum standards for classification as T3 and C2 biosolids). The solids that do not meet these criteria are defined as sewage sludge (EPA, Vic. 2004).

C2/T3: (Contaminant grade 2 and treatment grade 3) require management during land application to ensure protection of the environment, public health and agriculture.

Class A biosolids: Have undergone treatment to the point where the concentration of pathogens is reduced to levels low enough that no additional restrictions or special handling precautions are required by Federal regulations [40 CFR* Part 503]. If the Class A biosolids meet exceptional quality requirements for metals content, they may be sold in bags and applied in the same way as other soil conditioners such as peat moss.

Class B biosolids: Have undergone treatment that has reduced but not eliminated pathogens. By definition, Class B biosolids may contain pathogens. As a result, Federal regulations for use of Class B biosolids require additional measures to restrict public access and to limit livestock grazing for specified time periods after land application [40 CFR Part 503]. This allows time for the natural die-off of pathogens in the soil.

Contaminant grade: Grading category used to describe the quality of biosolids product based on the concentration of contaminants.

Critical nutrient concentration: The nutrient concentration in the plant or specified plant part below which the nutrient becomes deficient for optimum growth rate.

Effluent: liquid waste discharge such as residential and commercial sewage that has been treated to a quality suitable for a beneficial use.

Environmentally friendly goods: are goods produced, used or disposed of in a way that has a reduced or minimal impact on the environment.

Land disposal: Application of biosolids where beneficial use is not an objective. Disposal will normally result in application rates that exceed agronomic nutrient requirements or cause excessive contaminant accumulation in the soil.

Least significant difference (LSD): The difference required for one treatment to be statistically different from another at the 95% confidence level, expressed in identical units.

Lodging: When a standing crop is caused to lean or bend at 90 degrees due to adverse weather or soil conditions.

Soil test (analysis) deficiency critical level: That concentration of an extractable nutrient element below which deficiency occurs and above which sufficiency exists.

Soil test (analysis) toxic critical level: That concentration of an extractable nutrient element above which toxicity is likely to occur.

Sustainable use: The use of nutrients in biosolids at or below the agronomic loading rate and/or use of the soil conditioning properties of biosolids. Sustainable use involves protection of human health, the environment and soil functionality.

Tillers: shoots that sprout from the base of a grass.

Treatment grade: Grading category used to describe the quality of biosolids product based on a combination of defined treatment processes, microbiological criteria and stabilization to reduce vector attraction and odour generation.

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CHAPTER 1. INTRODUCTION

This thesis describes an innovative beneficial use of biosolids generated by Western Water at Surbiton Park (WWSP), Victoria, Australia. The study presents the findings from a two year field experiment in which canola and oats were grown as energy and fodder crops on biosolids amended soil. Two types of biosolids at various application rates were incorporated on a $40 \times 37 m^2$ plot of land at the waste water treatment facility at WWSP.

Seed and plant biomass responses of canola and oats to the applications of biosolids were evaluated under field conditions. Nutrients and heavy metals in soil and plants and pathogens in the amended soil were measured; leaching potential of NO_3-N was also investigated.

The effect of cropping sequence on nutrients and heavy metal residues accumulated after two years of successive applications of biosolids was also examined.

1.1. Biosolids under International context

Biosolids are treated or stabilized sewage sludges produced by a variety of treatment processes including biological nutrient removal, trickling filter and lagoon based systems (EPA Victoria, 2004).

Sewage sludge or biosolids production on a global scale is growing as a result of increasing human population and industrial activities. In Europe, North America, Australia, and New Zealand, the government and waste water authorities have given considerable attention on how to enhance the management of wastewater sludge and biosolids and improve efficiencies, maximize utilization of beneficial aspects, and reduce potential impacts of managing biosolids.

Table 1.1 shows the estimated sewage sludge production and population of various reporting countries; the data highlights that the higher-income countries have the most comprehensive infrastructure and treatment technologies (such as secondary and tertiary treatments) and produce the largest masses of wastewater sludge per person, whereas the middle-income countries included in Table 1.1 which have less-developed wastewater treatment infrastructure and collect and treat wastewater from lower percentages of their populations, produce far less wastewater sludge per person on a national level. This would imply steady increases in wastewater sludge production are likely in many parts of the world in the years to come.

Table 1.1 Estimated sewage sludge production and populations of reporting countries, 2008

Country	Estimated Sewage Sludge Production (dry metric tonnes)	Population
Australia/NZ	360,000	21,262,641
Brazil	372	188,078,000
China	2,966,000	1,313,974,000
Turkey	580	70,414,000
Slovakia	55	5,439,000
Hungary	120	9,981,000
Japan	2000,000	127,464,000
Canada	550	33,100,000
Italy	1000,000	58,134,000
Norway	86.5	4,611,000
Czech Republic	200	10,235,000
USA	6,514,000	298,444,000
Portugal	237	10,606,000
Germany	2,000,000	82,422,000
United Kingdom	1,500,000	60,609,000
Slovenia	57	2,010,000
Finland	150	5,231,000
Netherlands	1,500,000	16,491,000

Adapted from: Global atlas of excreta, wastewater sludge, and biosolids management: moving forward the sustainable and welcome uses of a global resource. United Nations Human Settlements Programme (UN-HABITAT), 2008. Edited by: Ronald J. LeBlanc, Peter Matthews, Roland P. Richard

Hence sewage sludge management is becoming increasingly an important environmental issue. There are various biosolids disposal techniques such as land filling, incineration and land application. Among these, agricultural land application is the most economical and environmentally attractive method of utilizing the nutrients from biosolids (Jeyakumar ***et al***. 2008).

Since biosolids contain significant quantities of organic matter, moisture, nutrients and trace elements; they are increasingly being viewed as a resource for agricultural and waste water treatment sectors. Moreover, the addition of biosolids to soil can also improve soil properties such as porosity, bulk density, aggregate stability and soil water holding capacity (Epstein, 2002). Biosolids can also be composted or heat dried usually at thermophilic temperatures (> 55^0C) to remove water and destroy pathogens and reduce odorous compounds. Heat drying and composting usually produces high quality biosolids which can be used primarily in agriculture as a soil amendment and are valued for the organic matter content. Heat-dried biosolids are also considered as a fertilizer. They may be used as a supplement to other inorganic fertilizer material to increase the plant nutrient content (Epstein, 2002).

The nitrogen content of composted biosolids is lower than heat-dried biosolids; hence, larger quantities can be applied to improve the soil physical properties.

Indeed, conventional agricultural activities that focus on cultivation and the use of chemical fertilizers expose soil to erosion by wind and water. Chemical fertilizers supply no organic matter; however biosolids can be used as a remedy to this agricultural practice by enhancing soil organic matter content and nutrient reserves in the soil. In addition to this, the importance of utilizing the nutrients in biosolids is emphasized by the fact that natural phosphorus (P) reserves are expected to be depleted by the end of this century (Leblanc *et al*. 2008).

1.2. Biosolids in Australia

Australia and New Zealand currently produce approximately 360,000 dry tonnes of biosolids annually. The bulk of sewage sludge produced in Victoria is stockpiled, with only five percent of the sewage sludge applied to land (DSE, 2003). In contrast, over sixty percent of sewage sludge produced in US is land applied, with one percent utilized in agriculture (U.S. EPA, 2002). The long term trend in US is towards sustainable beneficial reuse of sewage sludge as a fertilizer. Similarly, the EU policy also supports the enhanced use of sludge in agriculture (European Commission, 2001).

The cost of sludge/biosolids management is typically 40 to 50 percent of total capital and operating cost for wastewater treatment. The current average cost for biosolids management is in the order of $300/dry tonne, which equates to about $100 million per year.

Victoria alone has a stockpile of some 1.7 million dry tonnes of biosolids, and will cost about $500 million to manage (Gale, 2007). It is estimated that total annual production in Victoria is approximately 66,700 dry tonnes, with around 60 per cent of that production coming from the major metropolitan treatment plants (EPA Vic, 2004).

Hence, these stockpiles of biosolids need to be sustainably managed particularly for their nutrient and organic matter content.

1.3. Land application

Certainly, crop farming can reduce soil fertility, particularly in soil organic carbon and soil N (Haynes *et al*., 2003). Land application of biosolids has received increased attention in the last two decades (Robinson *et al*., 2002; During and Gath, 2002) and helps to replenish the reduced soil organic matter, provide nutrients such as N, P, S and essential micronutrients to plants and biosolids possess beneficial effects on microbial biomass and activity (Eriksen *et al*., 1999; Leifield *et al*., 2002).

The need to effectively deal with the disposal of sewage sludge and biosolids continues to be a challenge as the population increases. Land application of biosolids is becoming the favoured disposal option in many parts of the world because of the recognized benefits that biosolids can bring to soil. These benefits include increased organic matter content, improved soil structure and water infiltration (Morel and Guckert, 1981; Oberle and Keeney, 1994; Joshua *et al*., 1998; Johansson *et al*., 1999), and improved soil fertility and productivity (Peverly and Gates, 1994; Mosquera-Losada *et al*., 2001; Bhogal *et al*., 2003).

The application of biosolids to agricultural land increases the possibility of recycling valuable nutrients and organic matter and usually reduces the need for commercial fertilizers. It also reduces environmental and economic considerations that limit disposal in landfills, incineration or ocean dumping (Oliver *et al*., 2005; Singh *et al*., 2007).

Direct land application can be used beneficially in agriculture, forestry, and land reclamation. The biosolids can be applied either in a liquid form with low solids or as a semisolid following dewatering. Direct land application for beneficial use involves some form of partial stabilization such as digestion or alkaline stabilization (Epstein, 2002).

Australia has some of the most infertile soils in the world, as a consequence of the antiquity of the continent and extensive weathering of soils. This results in low levels of soil nutrients, particularly phosphorus. Historically Australian soils are deficient in P, N, K and some trace elements. Many agricultural soils are also deficient in both macronutrients (N, P and S) and micronutrients (Potter *et al*., 1999). Widespread low phosphorus levels have severely retarded biological activity in many soils; as a consequence, the shallow topsoil over large areas is low in organic matter and has poor structure. In addition, many of the sub soils have high clay contents, coarse structure and low permeability, and several contain appreciable amounts of sodium salts.

The agricultural system in Australian largely depends on the continued use of major and minor nutrient fertilizers to replace nutrients removed from the soil in harvested produce, lost via surface run-off or leaching or being bound to soil particles and made unavailable (NBRP, 2007).

Consequently, given the substantial amounts of biosolids annually generated by waste water treatment plants and the high cost of biosolids management, recycling and use of nutrients and organic matter contained in metropolitan wastewater biosolids in agriculture is potentially a better option from both an environmental and economic perspective.

However, biosolids usually contain high levels of P, N and heavy metals, which may either accumulate or leach in soils when applied at high rates (Bhogal *et al.*, 2003; King and Hajjar, 1990; MacLean *et al.*, 1987; McGrath, 1987). Elevated levels of heavy metals have the potential to cause environmental impacts, such as plant and microbial toxicity and food chain and ground water contamination (Bhogal *et al.*, 2003; Chang et al., 1987; Chaudri *et al.,* 2000; Dahlin and Witter, 1993; Zwarich and Mills, 1982). Hence, knowledge of the physical and chemical properties of biosolids applied to land is crucially important, particularly the total and the potentially bioavailable forms of nutrients and heavy metals.

The National Biosolids Research Program (NBRP)

Long term repeated agricultural land application of biosolids may increase heavy metals levels in the soil and plant system. Regarding the issue of heavy metals, various research projects began internationally since 1970s; however, the significance of this work to the existing Australian agricultural system was not clear. Moreover, the available relevant data on heavy metals risks from biosolids were confined to small number of sites in New South Wales, thus there was little evidence to substantiate whether these relationships (i.e. to use the data generated in NSW for other Australian soils and climatic conditions) were under-or-over protective across the various Australian soil and agricultural system. Because of these reasons, the National Biosolids Research Programme (NBRP) consisting of a coalition of seven research agencies around Australia was established in 2002 to coordinate the research activities across States relating to the benefits and risks of utilizing biosolids in agriculture.

Table 1.2 below summarizes the chemical properties and nutrient compositions of the various biosolids used across the States during the National Biosolids Research Program. As shown in Table 1.2 the concentration of Zn in the biosolids was higher than Cu across all the States with the exception of West Australian biosolids, in which high concentrations of Cu and nitrogen could possibly limit repeated application of biosolids in the long term.

Biosolids from Queensland had the highest total P level compared with the rest of the States which requires particular emphasis on P management. The pH of the biosolids ranged between 4.6 acidic in Victoria (East Gippsland and New South Wales) and 7.4 near neutral in South Australia.

Table 1.2 Selected chemical properties of the various biosolids used for the National Biosolids Research Program

Biosolids source and name	Sites applied	EC	pH	CEC	TC %	T N (%)	TP (%)	NH_4-N (µg/g)	NO_3-N (µg/g)	Cd_T	Cu_T	Zn_T
SA Bolivar air dried	SA	6.3	7.4	35	6.3	0.77	0.8	28	1690	1.8	315	435
SA Bolivar dried Lagoon	SA	7.0	7.4	28	8.6	0.98	1.0	49	1370	2.2	340	500
Vic Goulburn valley Water	Dookie	3.8	7.1	24	6.5	0.83	0.3	89	1420	1.4	65	180
Vic North East Water	Dookie	6.5	5.0	49	11.6	2.03	2.5	480	4010	0.9	100	300
Vic Dutson Downs Gippsland	Dutson	6.8	5.6	61	20.4	2.45	1.1	3280	3910	<0.5	70	180
Vic Dutson Downs East Gippsland	Dutson	4.1	4.6	21	10.6	1.25	0.3	82	2580	1.0	150	290
NSW Malabar STP	NSW	4.1	7.6	32	20.2	1.55	1.4	1480	104	5.4	420	650
NSW Bondi STP dewatered	NSW	5.9	6.2	37	28.7	2.50	1.1	3560	357	4.6	880	870
QLD Noosa	QLD	2.9	6.8	84	27.2	4.79	4.7	480	22	1.9	355	495
QLD Luggage point	QLD	7.6	6.6	68	32.8	5.72	2.7	4660	3	3.5	830	1705
WA Woodman point	WA	4.4	6.9	68	32.2	5.17	1.5	4520	4	2.0	1500	900
WA Beenyup WWTP	WA	4.3	6.8	60	34.7	5.54	2.0	4480	3	1.4	1170	615

EC= electrical conductivity expressed in dS/m, pH $CaCl_2$. CEC = cation exchange capacity (expressed in cmol (+)/kg and the subscripts “T” refers to total concentrations of Cd, Cu and Zn expressed in µg/g. Values indicate on dry weight basis

Adapted from the NBRP Draft position paper

Results of preliminary metal hazard assessment by the NBRP showed that Cd, Cu and Zn were the main metals of concern. Cadmium was chosen because of food chain concerns, whereas Cu and Zn were considerably higher in most biosolids and may affect environmental health. Thus, the NBRP determined critical soil concentrations for Cd, Cu and Zn that negatively influenced crop productivity and microbial processes across all NBRP sites. They suggested that the proposed heavy metal limits mainly depended on soil properties predominantly pH, organic matter, clay content and cation exchange capacity of a particular site.

Although, plant nutrients (N and P) were not the main focus of the NBRP, some experiments were conducted in the spring/summer growing seasons in Southern Queensland and indicated that the mineralization of biosolids were up to 3 times higher than the estimated rates of 15 and 25 % for anaerobically and aerobically digested sewage sludges, indicating rapid mineralization of N from biosolids (NBRP, 2007).

The Queensland field results were further verified and confirmed under laboratory conditions, that loss from denitrification was greater than losses from volatilization and much as 30 % of the N in biosolids was mineralized during the 3 months period after land application.

Regarding P management on biosolids amended land, the NBRP also suggested if biosolids are applied based on crop N requirement, excess P may accumulate and may result in a substantial off-site P movement in run-off or leachate contributing to eutrophication of aquifers.

Thus, for efficient management of N and P, the NBRP recommended that a national approach needs to be devised taking into account a range of soil properties and the various biosolids generated from different waste water treatment plants to improve the current biosolids guidelines and fertilizer advice.

Victorian component of the NBRP

With 66,700 dry tonnes biosolids produced annually, the Victorian water industry faces a major challenge to manage the biosolids. There is little space for expanding stockpiles and restrictions on landfill sites receiving biosolids and hence sustainable management biosolids is an issue that needs to be addressed.

As part of the (NBRP), Plant, soil and microbial measures from five biosolids and two metal salt trials were conducted in Victoria from 2003 to 2006 by the Victorian Department of Primary Industries in collaboration with the CSIRO (, NBRP, Vic. Component 2007).

The biosolids trials were conducted at Dutson Downs (cropping), Dookie (cropping), Melton (cropping), Pakenham (pasture) and Mildura (grape vines) which were planned to determine the effects of biosolid-derived nutrients and metals on the plant/soil system for a range of crops and soil types.

Biosolids were applied at six application rates at each trial site and compared to mineral fertiliser and control treatments. The biosolids application rates were computed using the annual nitrogen requirement of each crop and the nitrogen content of the biosolids (NLBAR). Soil and plant samples were analysed to determine crop production and the concentrations of nutrients and metals in the plant/soil system. Soil was sampled on two occasions; soon after the application of biosolids and at harvest. Field crops were sampled at mid-tillering (8-12 week growth stage) and at harvest. Pasture (ryegrass/clover) was sampled at seven targeted times over three years. The grapes were sampled from the vines annually.

Two metal salt trials were also conducted at Dookie and Dutson Downs. These trials were designed to investigate the response of plants and biota to three metals commonly found in biosolids; cadmium, copper and zinc. The soils were sampled after the metal salts were applied to the plots and at each harvest. Plants were sampled at mid tillering (8-12 week growth stage) and at harvest. As for the biosolids trial, plant and soil samples were analysed to determine yield, metal concentrations and microbial activity (NBRP, Vic. Component 2007).

Results of the findings showed that increased biosolids application rates increased phosphorus and nitrogen concentrations in soil as well as making the soil pH more neutral. These effects decreased over time. Overall grain yield in biosolids treatments decreased compared to the control but this may have been due to a lack of soil moisture in the grain development stage.

While biosolids generally had a negative effect on grain yields, at Dookie the recommended biosolids applications rate of 1 NLBAR resulted in increased dry matter and grain yield in years 1 and 3. This was also apparent at other rates. At Melton an increase in grain yields was observed in the final year at and above 1.5 NLBAR.

Soil microbial function was either stimulated or unaffected by the application of biosolids

In general, biosolids applications increased plant production and had little effect on soil microbial health at the five Victorian trial sites. The main metals investigated Cu, Zn and Cd increased in the soils and in crops at higher biosolids application rates but the concentrations were not enough to result in bioaccumulation or toxicity effects because of the low concentration of these metals in the biosolids. Some of the applied N and P was seemingly utilised by plants but the portion of N and P that are not utilised by the crop particularly in the higher application rates could either contribute to a pool of N and P in the soil or be exported and pose off site environmental concerns.

The Victorian component of the NBRP has not investigated the details of nutrient behaviour in biosolids amended soil particularly nutrient and metals behaviour under crop rotation regime, rather the NBRP nutrient work was focused on sites in Queensland (N and P) and in Western Australia (P), therefore this study focuses on nutrient (N, P and S) and heavy metals behaviour with emphasis given on crop rotation under the specific South Melton environmental conditions in Victoria(NBRP, Vic. Component 2007).

1.4. Western Water at Surbiton Park (WWSP)

Western Water is one of Victoria’s 13 regional urban water corporations which provide water and sewage services to over 53,000 properties and 134,810 people in an area of 3,000 square kilometers.

The region extends from Lancefield in the north to Melton and Rockbank in the south and from Myrniong in the west to Bulla in the east. It incorporates parts of Hume City Council and Melton, Moorabool and Macedon Ranges Shire Councils. Western Water’s region is a combination of urban and rural living. A significant proportion of the land is devoted to agricultural uses, particularly grazing and cropping.

Western Water operates four lagoon based and three mechanical treatment plants. Biosolids generated at the lagoon based treatment plants continuously accumulate in the lagoons with biosolids removal occurring as required. The mechanical recycled water plants continuously generate sludges which need to be managed on a daily basis (Figure 1.1).

The Western Water purification centre at Surbiton Park situated close to Melton treats mainly domestic effluent with only light industrial discharges to the plant (Western Water Environment, 2008).

The annual production of biosolids at WWSP in 2007/08 was 957 dry tonnes with 15-18 % solid content. Biosolids are categorized by EPA Victoria based on the contaminant concentration in the biosolids (contaminant grade) and the microbiological quality post treatment (treatment grade). The biosolids used in this project from WWSP were categorized as T3/C2 (treatment grade 3 and contaminant grade 2) (EPA, Vic. 2004).

Figure 1.1 Waste Water treatment facility at Western Water Surbiton Park (WWSP)

For the past 20 years, liquid sludge (approximately 10,000 m^3 with 2-3% solid content/year) generated by WWSP was disposed of in a controlled manner on a disposal site. However, there was evidence of an adverse impact on the groundwater at the disposal site due to these practices.

During 2007, WWSP began exploring options to cease disposal of liquid sludge. A sludge dewatering facility (belt press) was installed in December 2007, which converts the liquid sludge into a more solid form; currently the plant treats liquid sludge from the recycled water process to produce biosolids with low moisture content. The environmental benefits resulting from this facility include the reduction of a source of potential groundwater pollution through the production of a usable biosolids by-product with low moisture content and beneficial properties. However, biosolids generated at WWSP are being stockpiled and hence at present there is a strong need for alternative methods of utilization (Western Water Environment, 2008).

Western Water developed a biosolids policy in 2007 with the aim of maximising the beneficial use of biosolids by adopting sustainable and environmentally acceptable biosolids management practices. A Biosolids Reuse Strategy was also formulated in accordance with Western Water's Biosolids Policy and the relevant State and Federal policies to ensure that Western Water meets its commitment to 100% beneficial reuse of its biosolids for the 2008-2013 regulatory periods.

The strategy recognises Western Water's partnership with the Royal Melbourne Institute of Technology (RMIT University) (Western water Environment, 2008).

1.5. Significance of the study

The search for environmentally safe and economically feasible techniques of sewage sludge disposal is a challenge to wastewater treatment facilities.

Disposal options include landfill, incineration, ocean dumping and land application. Among the various options, beneficial use of biosolids through land application represents an environmentally and technically feasible alternative for managing biosolids (McFarland, 2001; USEPA, 2000). Because biosolids are rich in nutrients, land application is an efficient way to recycle these nutrients onto soils. Moreover, agricultural land application of biosolids has a lower capital investment than other biosolids management technologies such as surface disposal or incineration (USEPA, 2000). However, relatively high levels of potentially toxic trace metals such as Cu and Zn usually present in sludge from heavily urbanized and industrialized areas, can pose a risk to the environment unless well managed (Moolnaar, 1998; McBride, 1999).

Although, the major move toward sewage sludge management in Victoria has historically involved a progressively increasing stockpile, the Government and the water industry have acknowledged that this trend is not a sustainable management option (Reid, 2002). Not only is ongoing stockpiling contrary to obligations under the waste management hierarchy (reuse, recycling before disposal), it delays the costs associated with management since stockpile areas will eventually need remediation and rehabilitation.

The realization that continued stockpiling is not a sustainable management approach, led to the formation of a water industry-government working group coordinated by the Department of Natural Resources and Environment (NRE) to formulate a strategic, triple bottom line based approach for improving management practices (Reid, 2002).

At present, Australian National and State guidelines controlling metal contaminant concentrations in biosolids and biosolids-amended soils (NSW EPA, 1997; SA EPA, 1997; DPIWE, 1999; WA DEP, 2002; EPA Vic., 2004; NRMMC, 2004) are based on single values for all soil types and essentially follow European regulations and research (McLaughlin et al., 2000).

However, there are significant differences between European and Australian agricultural soils and Australian biota might be more or less sensitive to metal contaminants. European toxicity values to protect soil quality may thus be unsuitable for Australian soils, biota and climatic conditions.

To generate valuable information that can fit with the wider Australian agricultural context, the NBRP was established. However, while most of NBRP's work has focused on the derivation of soil quality guidelines for Cd, Cu and Zn as contaminants, they recommended that a national approach needs to be organized to effectively deal with N and P management on a biosolids amended land in order to develop guidelines for N and P management under different temperature, soil moisture and other climatic factors encountered under Australian conditions (NBRP, 2007).

Results obtained in the process of producing this thesis add to the knowledge of the behaviour of N and P in soils and plants and S in soil following land application of biosolids. It also includes heavy metals residues in soil and uptake by plants, where in this particular case, the biosolids used were dewatered biosolids and composted biosolids and the soil was a clay loam soil.

The realistic scale of the design of the field experiment enabled the generation of quality data specific to agronomic and environmental parameters. Thus, information generated from this research will be of relevance particularly to WWSP and to government and industries involved in land application of biosolids. It will also contribute some inputs to further refine the Victorian guidelines for land application of biosolids.

The findings of the study also add more critical information to appropriate research and development institutions working on agricultural land application of biosolids.

A major concern for sustainable utilization of nutrients from biosolids requires using nutrients efficiently and preventing off farm movement. Hence, there are considerable information gaps surrounding ways to improve the efficiency of the use of biosolids nutrients (Bell, *et al*., 2006). One of these gaps which is partially addressed in this thesis is the extent of nutrient leaching down the soil profile in an irrigated system.

Indeed, there is a considerable research conducted on the environmental effects of agricultural land application of biosolids, however, long term research on the usage of biosolids on crop land is still needed since many of its effects such as organic matter enrichment and the possible accumulation of toxic elements in the soil evolve slowly and are difficult to predict (Mc Grath, 1984; Bergkvist *et al*., 2003; Gaskin *et al*., 2003).

Australian and State guidelines are periodically being reviewed to incorporate new and relevant research findings and ensure the appropriateness of the document (Gough and Fraser, 1995; Barry *et al*., 1995; Gibson *et al*., 2002). In particular, no study has been reported in the literature on the impact of crop rotation in Australia on N, P and heavy metal accumulation in biosolids amended soil after crop harvest under field conditions. Moreover, the benefits of land application of biosolids to crop growers need to be evaluated on crop rotation basis (Karen *et al*., 1995) taking into account N, P and heavy metals residual effects on the subsequent crops (Binder *et al*.

2002). Certainly, biosolids are valuable resource and supply the necessary plant nutrients, hence the economic valuation of biosolids as environmental goods in the production of crops in biosolids amended soil needs to be investigated.

Hence, the literature suggests that there is a considerable research gap in Australia and particularly in Victoria with respect to nutrient and heavy metals accumulation in soils on which biosolids have been applied under a crop rotation regime. The experiments described in this study address a number of these issues.

Aim and objectives

The aim of this study was to investigate environmentally and economically sustainable and productive cropping management options for dewatered biosolids and composted biosolids from Western Water, Victoria. Canola and oats were chosen for this experiment, because the farmers around the Melton area grow canola for oil or energy production and oats for livestock fodder purposes. Growing these crops for energy and livestock production eliminates potential contamination issues.

Moreover, these crops have different rooting systems and can be rotated under the existing farming system around Melton and adjacent to the experimental site at Wester Water Surbiton Park (WWSP).

The specific objectives were to investigate:

- the effect of incorporating biosolids at various application rates on the seed, plant biomass, oil yield and yield components of canola and yield and yield components of oats under a crop rotation regime
- the optimum biosolids application rate to maximize crop production without contaminating the receiving soil with excessive nutrients and heavy metals in a clay loam soil
- the effect of application of biosolids on soil pH and EC of the amended soil
- the impact of land applied biosolids on bioavailability and accumulation of elements of concern in plants and in amended soil under field condition
- the impact of soil pH on heavy metals and P solubility in biosolids amended soil

- the effect of cropping sequence (canola/oats vs oats/canola) on the fate of elements in soil following biosolids application
- the level of pathogens (*E.coli*, *Clostridium perfrengens* and *Salmonella spp*) surviving in biosolids amended soil after crop harvest
- the economic value of biosolids as environmental goods in the production of canola and oats

Arrangement of the Thesis

The first chapter has presented a general introduction and overview of the research undertaken, whereas Chapter 2 focuses on the review of the various literatures available relevant to the research topic investigated. The review section focuses on the effect of using various types of sewage sludge and biosolids types on the nutrient and heavy metal status of a range of soil and plant types. It also describes the survival of pathogens in biosolids amended soil and plants. The laboratory procedures and the details of the field experimental design used for the two year period of study are presented in chapter 3 followed by soil and plant sampling procedures and the details of the analytical techniques employed to determine nutrients and heavy metals in soil and plants and the levels of pathogens. Chapter 4 evaluates and compares yield responses of canola and oats to varying applications of biosolids and it further describes the impact of biosolids applications on concentrations of canola seed oil.

Bioavailability and accumulation of total and extractable heavy metals in biosolids amended soil is the subject of Chapter 5. In this chapter, the uptake of heavy metals by canola and oats and their relationship with extractable soil concentrations is also presented.

The influence of biosolids applications on the levels of total and extractable N and P in soil and in plants is discussed in Chapter 6. This Chapter also explains the changes in the S status of the soil due to biosolids applications and further discusses the influence of cropping sequence on the levels of N and P residues left after two years successive applications of biosolids.

The assessment of pathogen in biosolids amended soil is presented in Chapter 7. Chapter 8 focuses on the economic assessment of the value of biosolids from the productivity and nutrient replacement perspectives. This chapter further discusses the optimum amount of canola oil that can be produced from a hectare of land at the optimum biosolids application rates and its estimated energy value.

2

CHAPTER 2. LITERATURE REVIEW

Introduction

This review is concerned with biosolids which are stabilized sewage sludge produced through the biological treatment of sewage sludge. Biosolids contain substantial amounts of plant nutrients, organic matter and micronutrients and are considered as a resource for the agricultural sectors.

Once treated to specified minimum standards they can be utilized for their nutrient, soil conditioning, energy, or other valuable properties. The solids that do not meet these minimum criteria are defined as sewage sludge (EPA Vic., 2004).

The review particularly focuses on the responses of soil and plants to the application of nutrients from anaerobically, aerobically digested dewatered biosolids, composted biosolids, fresh sewage sludge, heat treated biosolids, municipal solid wastes and other sludge types with some comparisons with conventional fertilizers under field and glass house conditions.

It also evaluates the responses of various crops to different biosolids applications in terms of plant biomass, nutrient uptake and the associated potential problems including pathogens, heavy metals and nutrients accumulated in organic wastes amended soil and plant system.

2.1. Historical perspectives of land application of Sewage sludge and biosolids

Land application of wastes has been a common practice since Biblical times (Deuteronomy 23:13 and Judges 3:20). The benefits of basic sanitation to society were realized by the Greek and Romans (Epstein, 2002).

In the 16th century, effluents were being used for crop production in Bunzlau, Germany. This practice began in 1550 and continued for 300 years (De Turk, 1935). Jewell and Seabrook, (1979) cited Gerhard, (1909), who indicated that the earliest documented sewage farm or sewage irrigation system, was in Bunzlau in 1531.

In 1859, in England, a Royal Commission on Sewage Disposal proposed the application of town sewage to land as a means of keeping it away from the river and hence preventing river pollution (Webber and Hillard, 1974). Another early sewage farm was established in Edinburgh, Scotland around 1860. By 1876, 35 towns in Britain used land treatment. Large sewage farms were established in Paris, France (1869) and in Berlin, Germany (1874).

The production of crops using sewage sludge irrigation was also practiced as early as 1872 in Augusta, Maine (Jewell and Seabrook, 1979).

Some limited land application was practiced after the beginning of sewage treatment in the 1880s and utilization of sewage sludge as organic amendment was recognized after the initiation of sludge activation processes (Noer, 1926).

Anderson (1959) stated that the use of sewage sludge as a fertilizer in USA dates essentially from 1927, when a large activated sludge treatment plant went into operation for the city of Milwaukee, Wisconsin demonstrating the utilization of biosolids as fertilizer from 1930 to 1957.

2.2. Waste water treatment processes

Waste water treatment plants use a combination of primary, secondary and tertiary wastewater treatment processes. The solids from these three stages are sent to a digester to allow anaerobic bacteria to break down the organic sludge into methane and other gases reducing pathogens and offensive odours. This makes the sludge less attractive to flies and insects. Digested sludge is withdrawn from the bottom of the digester into the sludge holding tanks. The sludge holding tanks store the digested sludge until it can be pumped and transported for various uses.

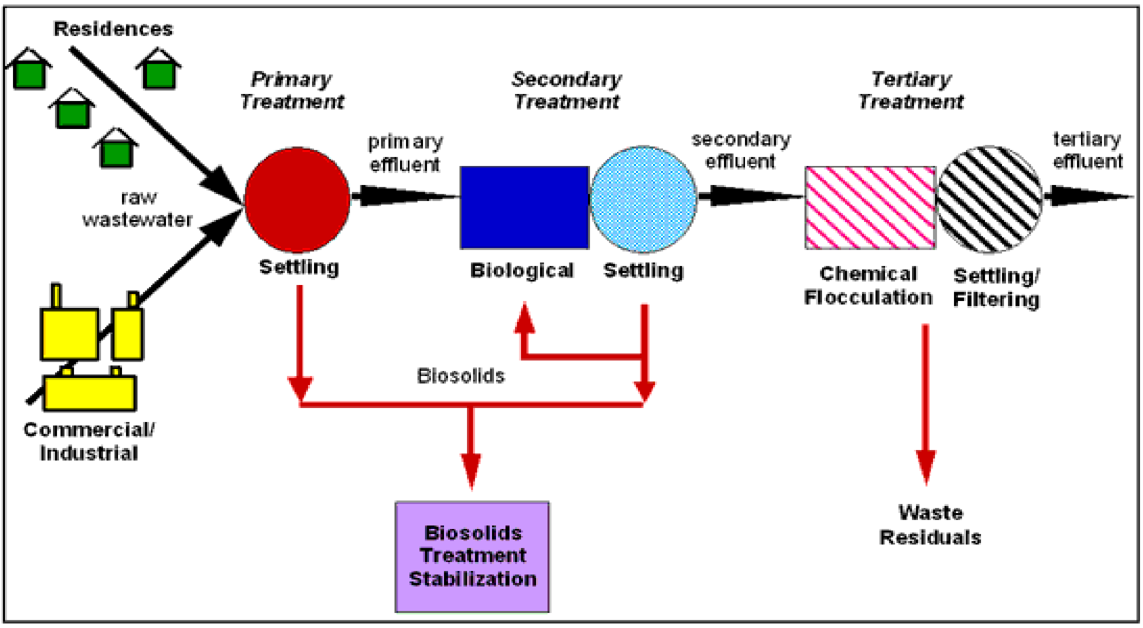


Figure 2.1 The various waste water treatment processes and biosolids production stages employed in waste water purification centers.
Source: http://www.nps.gov/plants/restore/pubs/biosolids/what.htm

Further treatments includes various stabilization techniques such as aerobic and anaerobic digestion, alkaline stabilization, composting and heat drying which increase the quality and beneficial uses of biosolids particularly for land application and other uses (Western Water, 2003a). Figure 2.1 shows the different waste water treatment stages and the production of biosolids.

2.3. Biosolids classification

In Australia, classification of biosolids is based on two criteria, these are, the concentrations of contaminants in the biosolids and the microbiological quality after passing the treatment processes. Thus biosolids are classified as contaminant grade (C1 or C2) and treatment grade (T1, T2 and T3) which are derived from the treatment technology employed, microbiological norms and measures used to reduce bacterial regrowth, vector attraction and odour (EPA Vic., 2004).

Table 2.1 shows the chemical contaminants limits for C1 and C2 biosolids, whereas Table 2.2 depicts the microbiological criteria used to classify biosolids according to EPA Victoria. The categorization of biosolids based on treatment and chemical grade and their allowable end uses are also summarized in Table 2.3

Biosolids can also be classified as unrestricted and restricted, with the restricted material (e.g. C2/T1, C1/T1, and C2/T3) entailing management during land application to ensure protection of the environment, public health and agriculture.

Table 2.1 Chemical contaminant limits for C1 and C2 biosolids and the ceiling concentration Limits for biosolids the receiving soil (µg/g).

Chemical Contaminant	Maximum Limit in Grade C1 biosolids (µg/g) and Maximum for Receiving Soil Contaminant Limit	Maximum Limit in Grade C2 biosolids
Arsenic (As)	20	60
Cadmium (Cd)	1	10
Chromium[1] (Cr)	400	3000
Copper (Cu)	100 (150)[2]	2000
Lead (Pb)	300	500
Mercury (Hg)	1	5
Nickel (Ni)	60	270
Selenium (Se)	3	50
Zinc (Zn)	200 (300)[3]	2500
DDT,DDE, DDD	0.5	1
Organochlorine pesticides	0.05	0.5
PCBs	0.2	1

Source: Victorian Environment Protection Authority, Guidelines for Environmental Management, Biosolids Land Application, EPA, Victoria, (2004).

The superscripts 1 refers to Chromium (III) limit due to expectation that this will be the dominant form, 2 stands for Cu concentrations of 150 mg/kg limit for biosolids products composted to AS 4454 and 3 refers to Zn concentrations of 300 mg/kg zinc limit for biosolids products composted to AS 4454.

Table 2.2 Classifications of biosolids based on microbiological criteria and the maximum EPA Victoria recommended ceiling limits for *E.coli,* Salmonella and Enteric viruses under each treatment grade category (values on dry weight basis)

Treatment Grade	Pathogens		
	E.coli	Salmonella	Enteric viruses
T1	< 100 MPN /g	< 1/ 50g	≤ 1 PFU/ 100 g
T2	< 1000 MPN /g	< 10/ 50g	< 2 PFU/ 10 g
T3	< 2000,000 MPN /g	-	-

The values shown above are used for verification and routine monitoring.
MPN and PFU refer to maximum probable number and plaque forming units
Adapted from Guidelines for Environmental Management, Biosolids Land Application, Environment Protection Authority, Victoria, 2004

Table 2.3 Classification of biosolids based on treatment and chemical grade and their permissible end uses

Treatment grade	Chemical grade	Unrestricted	Restricted Uses					
			Agricultural Uses			Non-Agricultural Uses		
			Food crops consumed raw in direct contact with biosolids	Dairy and cattle grazing/fodder (poultry), food crops consumed raw but not in direct contact	Processed food crops	Sheep grazing and fodder (horses, goats), on food crops, woodlots	Landscaping (unrestricted public access)	Landscaping, (restricted public access), forestry, land rehabilitation
T1	C1	√	√	√	√	√	√	√
T2	C1	X	X	√	√	√	√	√
T3	C1	X	X	X	√	√	X	√
T1	C2	X	√	√	√	√	√	√
T2	C2	X	X	√	√	√	√	√
T3	C2	X	X	X	√	√	X	√

The symbol √ refers to the biosolids grade that will generally be acceptable for the end use. Biosolids grades less than T1C1 will be subject to management controls, whereas X indicates the biosolids of this quality are not acceptable for the end use (would require a risk assessment and site specific EPA approval/licensing).
Adapted from Guidelines for Environmental Management, Biosolids Land Application, EPA Victoria, 2004

Contaminant grade C1 biosolids are the highest quality biosolids with low contaminant levels and specific management control by the end users is not required. The C1 limit has been derived for individual contaminants based on a conservative setting-protecting the most sensitive land use from the application of biosolids as a complete topsoil replacement. As a result, the C1 limit is adopted from the most rigorous soil investigation values for protection of human health in residential areas, for instance protecting children who come into contact and ingest soil according to the Health Investigation Level (HIL) in the National Environment Protection Measure (NEPM 1999). The C2 limit offers the maximum ceiling concentration above which contaminant levels are regarded as excessive (EPA Vic., 2004).

2.4. Physicochemical characteristics of biosolids

Since the character and quality of biosolids determine their use for land application, the physical characteristics of biosolids are categorized into three categories: physical, chemical, and biological. Physical properties affect the method of application, as well as the soil's physical and chemical properties. Several of these physical properties have an important effect on plant growth. They can affect the availability and accumulation of plant nutrients and trace elements. The important physical characteristics are solid content, particle size and moisture content.

Chemical properties have an effect on plant growth as well as the soil's chemical, biological and physical properties. The important chemical characteristics include pH, soluble salts, plant micro and macro nutrients, organic chemicals, essential and non-essential trace elements to humans and animals. The chemical properties of biosolids are determined by several factors which includes quality of waste water, extent of treatment (primary, secondary, tertiary), use of chemicals (ferric chloride, polymers etc) and method of stabilization (e.g. lime treatment) (Epstein, 2002).

Biological properties effect the soil's microbial population and decomposition of organic matter in soil, the environment and human health (Epstein, 2002). Biosolids contain different types of microorganisms; several of them can be beneficial whereas others may be harmful to humans, animals and plants.

The solids content of biosolids influences the method of land application. Liquid or low-solids biosolids will generally be injected into the soil to prevent vectors and provide better aesthetics. The addition of liquid biosolids also increases the moisture content of the soil, which could benefit plant growth. The organic matter content is diluted and consequently its benefit in improving soil structure will occur only after repeated applications and after a long period of time. The amount of plant nutrients and trace elements depends on the quantity and percent solids of the biosolids. Dewatered or semisolid biosolids are usually spread on the surface and subsequently ploughed into the soil. The concentration of solids adds organic matter to the soil. This added organic matter improves the soil's physical properties, especially soil structure, soil moisture retention, soil moisture content, and cation exchange capacity of the soil (Epstein, 2002).

2.5. Effect of biosolids application on soil temperature and seed germination

The addition of organic matter from biosolids may possibly affect soil surface temperatures and in the spring hasten germination of crops. High solid biosolids are usually compost or heat-dried products which are an excellent source of organic matter. They also increase soil water retention, available water to plants, soil water content, water infiltration and permeability and hence could contribute to increases in soil temperature and germination and a reduction in soil erosion and run off (Epstein, 1997).

Well stabilized composts are usually differentiated by the absence of chemicals that slow down seed germination and plant growth performance (Roe, 1998). Fresh composts usually possess some damaging organic acids such as acetic acid, acetaldehyde, ethanol, acetones and ethylene which have severe toxicity to plants (Ozores- Hampton, 1998). Germination of crops can be slowed down due to high levels of salts and the discharge of these organic acids into the compost. Plant toxicity effects can be examined by conducting germination tests (Gariglio ***et al***., 2002; Brewer and Sullivan, 2003).

Giuseppe and Giovanni, (1993) investigated the effect of digested sewage sludge application at a rate of 0.5, 1 and 2 kg/m^2 and commercial fertilizer on the germination and yield of peas and broad bean under field condition. They reported that relative to the control plots, the presence of sludges in the soil improved germination and yield of both species to the same extent as did the fertilizer.

Murillo ***et al***., (1995) evaluated the effect of two urban composts on germination of ryegrass and sunflower and seedling performances in pot experiment. From their findings they concluded that compared with the control, coarse matured urban compost treatments (100%) increased the germination index of the crops as a result of enhanced root development.

Staden *et al.*, (2005) conducted a laboratory experiment to investigate the effect of different temperature and watering frequencies on seedling growth in Ornithogalum longibracteatum and Tulbaghia violacea plants. Thirty-day-old seeds were incubated to constant temperatures (10, 20, 25, 30, 35 and 40 ^{0}C)) and alternating temperatures (25/20 and 30/15 ^{0}C) under a 16-h light:8-h dark photoperiod with a photosynthetic photon flux density of 45.6 ± 5 μmol m^{-2} s^{-1} provided by cool-white fluorescent lamps. Germination counts for all experiments were made daily for 25 days. They observed increases in the germination percentage of O. longibracteatum and T. violacea seeds at 25 ^{0}C and decreases in germination for temperature greater than 25 ^{0}C.

Low temperatures caused poor germination in both species. Optimum percentage germination was attained between 20 and 30 ^{0}C. Low temperatures are conducive for bulb formation and high temperatures for initiation of leaf growth. Joanna and Wester, (2004) investigated the effect of applying 34 t/ha biosolids on soil water, soil temperature, and seedling emergence and growth of blue grama (Bouteloua gracilis) and green sprangletop (Leptochloa dubia) in a Chihuahuan desert grassland. Greenhouse and field experiments were conducted for 2 years. Biosolids did not affect mean soil temperature (at seed depth) but usually increased minimum and reduced maximum temperatures. Biosolids generally reduced soil water loss. They concluded that when environmental conditions are neither extremely unfavorable nor extremely favorable, the presence of surface-applied biosolids and the resulting reduction in soil water evaporation and moderation of soil temperature extremes may provide conditions conducive for successful emergence.

Moral et al., (2006) studied the effect of applying composted sewage sludge in a growth medium for broccoli (Brassica oleracea var. Botryti cv. Marathon). Increasing quantities of composted sewage sludge to peat (0 %, 15 %, 30 % and 50 %, v/v) were used. Their results showed that using composts even at 50 % mixing rate for the preparation of substrates for the propagation of broccoli was sufficient

The literature suggests that some experiments conducted to investigate the effect of biosolids applications on the germination of seeds produced contradictory results; in some cases various researchers reported either an increase or a decrease in germination of seeds of different plants due to biosolids and compost applications, whereas others found no effect. Whether biosolids have any effect on seed germination seems to depend on several factors, among these the nature and characteristics of soil and biosolids, plant species, and the quantity and method of application are; however, this needs to be investigated under field conditions.

2.6. Potential problems associated with land application

2.6.1. Pathogens in biosolids

A pathogen is an organism or substance capable of harming public health or causing disease (USEPA, 1999). With regard to human health, the economically most important bacterial pathogens include Salmonella sp., *E. coli* O157 H7, and *Clostridium perfringens*. Salmonella are Enterobacteriaceae which are commomly found in the environment and include more than 2000 serotypes. These pathogenic bacteria are found in waste water and include the different serotypes which cause typhoid, paratyphoid fever and gastroenteritis. At any given time, 0.1% of the population excretes Salmonella which produce an endotoxin that causes fever, nausea and diarrhoea and could be serious if not well treated by antibiotics (Bitton, 1994).

Salmonella paratyphi and *Salmonella typhimurium* are serotypes mostly associated with food contamination. *E.coli* is a species that occurs normally in the intestines of man and other vertebrates and is ubiquitous. Some species can be pathogenic. *E. coli* O157:H7 is a Vero toxin-producing strain and was recognized as a pathogen in 1982 (Boyce *et al*. 1995). Some toxin producing *E. coli* strains have also been implicated in diarrhoea, hemorrhagic colitis, and the hemolyticuremic syndrome. It is important to measure the survival of *E. coli* in biosolids, since it is found in biosolids and with high possibility for regrowth (Straub *et al*., 1993).

Clostridium perfringens is an anaerobic, gram-positive, sporeforming, and heat-resistant bacterium. Municipal sludges contain high levels of *Clostridium perfringens* and hence this organism can be used as an indicator for the survival of pathogens in sludge treatment processes (Salsali *et al*., 2007).

Recycling of biosolids to agricultural land provides as an important management technique for sewage sludge and other sludges (USEPA, 1999; Zhou, 2002; Li & Wu, 2003), however the public has concern regarding pathogens when biosolids are applied to land (Deportes et al., 1995; Sahlstrom, 2003). Pathogens entering the soil during land application of biosolids can survive in the soil for two months or more (USEPA, 1994). Temperature, moisture, pH, soil composition and the presence of other microorganisms are some of the factors that determine the survival of bacteria in the soil (Mawdsley *et al*., 1995; Garrec *et al*., 2003).

Due to the increased knowledge of the importance of enteric disease transmission from agricultural land management practices, the microbiological quality of biowastes applied to land has become a main concern of the food supply industry (Pennington, 2001).

To reduce the number of pathogens in biosolids, the most frequently employed sewage treatment techniques include digestion, alkali-stabilization, composting, irradiation and pasteurizations (USEPA, 1994; Ho *et al*., 2000). Biosolids should better be used in dry weather conditions to reduce the risks of faecal coliforms to water resources.

WWT and removal of pathogens from biosolids

The purpose of wastewater treatment is to reduce pathogens and sanitize effluent prior to discharge into water courses. The efficiency of removal differs with the different unit processes depending on the organisms and their physical and biological properties. For instance, many parasites survive the wastewater treatment process and accrue in the solids fraction (sludge), as a result of their densities (Farrell *et al*., 1996). Pathogen type and densities in biosolids is mainly a function of the wastewater and biosolids treatment processes.

During wastewater treatment, pathogens settle in the sludge produced by the separation of solids from wastewater (Fradkin *et al*., 1989; Lasobars *et al*., 1999). Thus, the number of pathogens in biosolids can be higher than in wastewater (Nell *et al*., 1983). Moreover, many microorganisms are known to survive better in wastewater when they are associated with the solid particles rather than in suspended state. Hence, it is likely that these microorganisms will survive longer in biosolids (Scheuerman *et al*., 1991; Straub *et al*., 1992).

The solids resulting from wastewater treatment should undergo further treatment prior to land application. Land application of biosolids requires the disinfection and stabilization of biosolids. The aim is to reduce the level of pathogens, reduce vector attraction and produce a stabilized product that will not putrefy very rapidly or produce unpleasant odours.

In order to eliminate the risk factors different technologies are used, thermophilic aerobic stabilization with or without a subsequent anaerobic process, lime disinfection of sludge by $Ca(OH)_2$ or CaO, composting of sludge, and others (Plachy, 1995; Novak, 1994).

Aerobic digestion is performed under mesophilic conditions ranging in temperature from ambient to 15-37 ^{0}C and retention times of 10 to 20 days, whereas, anaerobic digestion is performed under either mesophilic or thermophilic conditions. Mesophilic anaerobic digestion is usually attained at temperatures 30 ^{0}C to 38 ^{0}C, whereas in thermophilic anaerobic digestion the temperatures range from 50 ^{0}C to 60 ^{0}C (Epstein, 2002).

Nonetheless, many types of pathogens can survive such treatments (Watanabe ***et al***., 1997; Carrington ***et al***., 1982) and higher temperatures are often needed to improve the rate of sludge stabilization and to increase the reduction of pathogens.

The enhanced pathogen reduction observed in staged thermophilic, temperature phased and 2-phase digestion technologies is generally attributed to elevated temperatures (Schafer and Farrell, 2000). However, in many such processes pathogens are also exposed to higher concentrations of organic acids and the effectiveness of organic acids is believed to be dependent on concentration, pH, temperature, exposure time and the degree of sensitivity of specific types of pathogens (Goepfert and Hicks, 1969; Abdul and Lloyd, 1985; Fukushi ***et al***., 2003). These factors, either alone or in combination, have an impact upon the extent of injury of microorganisms in anaerobic digestion. The level of injury varies and will be a function of the specific microorganism's susceptibility.

Alkaline stabilization (lime treatment) of biosolids was accepted as a method of deodorizing and disinfecting the material. According to USEPA 503 regulations requirement the pH of biosolids should be increased to 12 for a minimum of 2 hours (USEPA, 1992). Since ammonia is liberated during the addition of lime, it acts as a disinfectant. If the addition of lime is inadequate to keep the pH for the time required to disinfect the biosolids, the pH will decline and surviving bacteria will grow when conditions become favourable **(**Epstein, 2002).

A number researchers including Niewolak and Szelagiewicz (1997); Novak (1994) and Plachy (1995) have indicated that composting causes significant decreases in pathogenic bacteria, fungi and helminth eggs and produces high-quality organic manure with a considerable portion of humic substances. Wiley (1962) stated that pathogen destruction during composting is the result of thermal kill and antibiotic action, or the decomposing organisms or their products.

Salmonella inactivation rates are generally high during the various wastewater treatment processes (Sahlstrom ***et al***., 2004), but it is known to survive in low numbers (0.45–7.5 cells g^{-1}) in biosolids (Watanabe ***et al***., 1997). Salmonella can survive up to 3 months in stored slurries, whereas a low survival time of one month has been reported in biosolids applied to land (Nicholson *et al*., 2005). Regrowth of *Salmonella* occurs under certain conditions in stored biosolids (Hussong *et al*., 1985; Gibbs *et al*., 1997), bagged biosolids based products (Skanavis and Yanko, 1994), composted biosolids (Sidhu *et al*., 2001) and soils treated with biosolids (Zaleski *et al*., 2005), which adds to its unpredictable behaviour in biosolids. Prolonged survival for more than 6 months can be expected in winter **(**Avery *et al*., 2004).

Regrowth of *E. coli* O157:H7, like other enteric bacteria in biosolids or sludge applied to the land, is also possible under certain conditions. The actual numbers of *E. coli* O157: H7 in sewage and its survival during wastewater treatment of is still unknown due to paucity of reported data.

The correlation between the reduction of indicator bacteria and pathogens during biosolids treatment processes is also conflicting**.** Gibbs *et al.* (1994) reported no correlation between the reduction in faecal coliforms, faecal streptococci and *Salmonella* numbers during anaerobic digestion. Sorber and Moore (1987) found higher inactivation rates of *Salmonella* in biosolids amended soil as compared to faecal indicators. Eamens *et al*. (1996) reported that there is no correlation between *Salmonella* die-off and decline in *E. coli* or FS numbers during the storage of biosolids or in biosolids amended soils.

Horan *et al*. (2004) investigated enteric bacteria pathogen die-off during mesophilic anaerobic digestion in UK and reported that for all the pathogens evaluated, die-off by compliant mesophilic anaerobic digestion is in excess of the numbers routinely encountered in sludge and thus, recommended that existing assets in the UK which utilise compliant mesophilic anaerobic digestion should meet the regulatory requirements for pathogen reduction .

Smith *et al*. (2005) investigated the factors controlling pathogen destruction during anaerobic digestion of biowastes, results of the experiment demonstrate that *E. coli* and *Salmonella* spp. are not damaged by mesophilic temperatures, whereas rapid inactivation occurs by thermophilic digestion and concluded that efficient mixing and organic matter stabilisation are the main factors controlling the rate of inactivation under mesophilic conditions and not a direct effect of temperature per se on pathogenic organisms.

Pepper et *al*. (2008) evaluated the fate and transport of potential biological and chemical hazards within biosolids, and the influence of long-term land application on the microbial and chemical properties of the soil. Direct risks to human health posed by pathogens in biosolids have been shown to be low. Risks from indirect exposure such as aerosolized pathogens or microbially contaminated ground water are also low. The study concluded that long-term land application enhanced microbial activity and no adverse toxicity effects on the soil microbial community and hence long-term land application of Class B biosolids is sustainable.

Gerba and Nwachukuto (2008) reviewed the occurrence and persistence of *E. coli* O157:H7 in surface and ground waters, wastewater and animal wastes and in soil and sediments, and reported that *E. coli* O157:H7 can be expected to be present in any waters in which *E. coli* is detected. Even though, colonized cattle appear to be the main source of the organism in the environment, many warm-blooded animals (livestock, pet anaimals and birds) can also carry the organism. Although the concentration of pathogenic *E. coli* at times is high in surface waters (i.e., after storm events), its lower infectivity results in risks less than that of enteric viruses and protozoan parasites

E. coli O157:H7 may persist in the environment for days to weeks depending on environmental conditions and Gerba and Nwachukuto (2008) concluded that in some environments rich in organic matter, pathogenic stains may actually increase in numbers.

Tanner *et al*. (2008) investigated the occupational risk of pathogens from bioaerosols generated during land application of class B biosolids. More than 300 air samples were collected downwind of biosolids application sites at various locations within the United States. Coliform bacteria, coliphages, and heterotrophic plate count (HPC) bacteria were counted from air and biosolids at each site. To estimate exposure to Salmonella and enteroviruses in air, concentrations of coliforms relative to Salmonella and concentrations of coliphage relative to enteroviruses in biosolids were used, in conjunction with levels of coliforms and coliphages determined in air during the study.The results showed that the heterotrophic plate count bacteria were ubiquitous in air near land application sites whether or not biosolids were being applied and the concentrations were positively correlated to windspeed, whereas, coliform bacteria were detected only when biosolids were being applied to land. Environmental parameters (wind speed, temperature and humidity) had little impact on concentrations of microorganisms in air immediately downwind of land application. From the findings, it was concluded that the occupational risk of infection from bioaerosols was estimated to be 0.78 to 2.1%/yr. Worst case exposure scenarios carried an estimated annual risk of infection of up to 34%, with viruses posing the greatest threat. Based on previously reported literature, risks from aerosolized microorganisms at biosolids land application sites seem to be lower than those at wastewater treatment plants.

Factors affecting the survival of pathogens during biosolids production

The inactivation of pathogens in biosolids depends upon a number of factors such as temperature (Martin ***et al***., 1990; Gerba, 1986**),** moisture content **(**Ward ***et al***., 1981; Russ and Yanko, 1981) and competition from indigenous microflora (Hussong ***et al***., 1985; Sidhu ***et al***., 2001; Pietronave ***et al.***, 2004). Factors such as predation, pH, sunlight and oxygen also influence pathogen inactivation in biosolids. The degree to which these factors influence survival of pathogens can vary from pathogen to pathogen and with the type of sludge treatment.

In laboratory experiments, reduction in temperature and increase in solids content has been shown to enhance pathogen survival (Feachem ***et al***., 1983).

Certain chemicals produced during digestion are toxic to some bacteria. Kunte ***et al***. (1997) reported that concentrations of high volatile fatty acids in anaerobic digesters significantly decreased the pathogen population. According to Strauch (1987), 90 % of *Salmonella* reduction is related to pH decrease in the substrate. The decrease in the pH value during storage is influenced by the natural bacterial flora producing fatty acids which have toxic effects upon Salmonella.

It is conceivable that the bacterial population would recover if these toxicants and inhibitors were removed as a result of the centrifugal dewatering process. Flocculant polymers used to condition the biosolids prior to dewatering are partially biodegradable and hence may supply nutrients or other chemical factors with impacts on bacterial growth (Chang ***et al***., 2001).

Furthermore ammonia is known to be bactericidal. During composting, ammonia (NH_3) is volatilized and is toxic to numerous bacteria and viruses (Ward and Ashley 1976; Taylor ***et al***., 1978;). Sikora ***et al***. (1985) also reported that NH_3 was virucidal in biosolids and in ammonium chloride solutions. Heat increases the rate of viral inactivation by NH_3 in sludge (Ward and Ashley, 1976).

Following the criteria established by the U.S. Environmental Protection Agency municipal sludge rule (40 CFR Part 503 Rule) Ponugoti ***et al.*** (1997) evaluated the effectiveness of various treatment processes in decreasing the density of pathogens municipal sludge. Biosolids samples were taken from different waste water treatment systems and analysed for indicator and pathogenic organisms. Results of the study showed that anaerobic digestion was superior to aerobic digestion in lowering the density of pathogens levels under the given field conditions. Composting produced better-quality product than the products produced under anaerobic and aerobic digestion. It was concluded that the Class B requirements under the 503 Rule are practical and achievable by most existing treatment systems, while the Class A requirements under the same rule might not be easily achieved by many existing waste water treatment facilities.

The volatile suspended solids (VSS) loading rates determine the log reductions in fecal coliform and fecal streptococci under anaerobic condition. Conversely, such trend was not obvious in aerobic digestion systems. Salmonella density reductions did not appear to be dependent on VSS loading rates in either case (Ponugoti *et al.* (1997).

Piterina *at al*. (2010) examined the pathogens in sludge at various stages during and following autothermal thermophilic aerobic digestion, for more than a year. The findings indicated that

Pathogeni*c Salmonella* spp. and fecal-coliform indicator densities were less than the limits used to certify class A biosolids in the final product. The autothermal thermophilic aerobic digestion deactivated the enteric pathogens present at the inlet during the digestion process and was not detected in the final product.

Surampalli *et al.* (1993) evaluated the pathogen and indicator bacterial levels in the sludge samples taken from two wastewater-treatment plants. The treatments plants are extended-aeration and oxidation-ditch-type plants with no aerobic or anaerobic digestion facilities following the secondary treatment except for sludge-storage facilities. The sludge samples were analysed to determine the sludge pathogen and bacterial reductions in aerobic digestion and in storage, inaddition to fecal coliforms, fecal streptococci, and *Salmonella,* total suspended solids, volatile suspended solids, pH, and temperature were also measured. The findings suggest that a two-order pathogen reduction was observed, it was concluded that the improved pathogen reduction was due to the design conditions of the plant, large sludge age, and long detention times.

Using a moderate aeration during the composting process in a semi-industrial pilot plant Hassen *et al.* (2001) investigated pathogen bacteria, fungi, fecal indicator bacteria: total coliforms, faecal coliforms and faecal Streptococci, present in a compost of municipal solid waste. The results showed that due to auto-sterilization created by relatively high temperatures (60–55°C), significant reduction in bacterial communities were observed, including Escherichia coli and faecal Streptococci populations, yeasts and filamentous fungi, however bacterial spores population increased at the start of the composting process, but after the third week their number was reduced significantly.

On the 25^{th} day of composting when the temperature reached 60°C, Salmonella was not observed however, the bacterial population increased gradually during the cooling phase. During the mesophilic phase and at the beginning of the thermophilic phase, Staphylococci seemed to be the dominant bacteria; bacilli predominated during the remainder of the composting cycle.

It was reported that the emergence of gram-negative rods during the cooling phase may signify a severe risk for the sanitary quality of the final product intended for agronomic reuse. However, compost sonication for a 3 min induced the inactivation of delicate bacteria, in particular gram-negatives. On the other hand, gram-positive bacteria, especially micrococcus, spores of bacilli, and fungal propagules survived, and were high in number in the final composted product.

Composting is frequently used as an efficient way of stabilizing wastewater biosolids and lowers the pathogens to very low concentrations. However, under certain conditions *Salmonella typhimurium* can regrow in previously composted biosolids.

Using sterile and non-sterile composted biosolids, Sidhu *et al*. (2001) monitored the growth of seeded Salmonella typhimurium in composted biosolids for period ranging from two weeks to two years maturity. The results indicated that seeded *Salmonella typhimurium* colonized rapidly in sterilized biosolids and reached maximum population density. On the contrary, growth of seeded *S. typhimurium* was suppressed in non-sterilized compost, the result showed that there was a significant decrease decline in the growth rate of seeded Salmonella in sterilized compost when the compost was stored, suggesting that bio-available nutrientsdecreased with storage. Conversely, in non-sterilized compost this was not the case, suggesting that indigenous microflora may contribute a considerable role in suppressing the regrowth of salmonella in composted biosolids.

When *salmonella* inactivation rate for two week period of storage was compared with the inactivation rates for two years period, the inactivation rate in the two weeks period was seven times higher than the compost stored for two years, suggesting that the antagonistic effect of indigenous microorganisms towards *Salmonella* declined with compost storage. It was concluded that Salmonella can regrow in all composted biosolids, nevertheless, the indigenous microflora can significantly reduce the regrowth potential and therefore long-term storage of compost is not recommended since it will possibly increase the pathogen regrowth potential.

To assess the effect of volatile fatty acids on the inactivation of *Clostridium perfringens* over a range of digestion procedures, Salsali *et al.* (2007) conducted an experiment, by adding an equimolar mixture of acetic, propionic and butyric acids to the digester effluent for a period of 24 h at temperatures of 35 ^{0}C, 42 ^{0}C, 49 ^{0}C and 55 ^{0}C. They reported *Clostridium perfringens* inactivation in digester effluents, when dosed with volatile organic acids, was found to depend on pH, acid concentration and temperature. The temperature above 55 ^{0}C appeared to enhance the inhibitory affect of the organic acids at higher concentrations, suggesting high concentration of organic acids at pH value of 4.5-5.5 during thermophilic digestion substantially reduces concentrations of *Clostridium perfringens* in municipal sludge.

Table 2.4 below summarizes the pathogens *E.coli*, *Salmonella* and the indicator *Clostridium perfringens* in wastewater and sludge, whereas, Table 2.5 depicts pathogen inactivation during waste water treatment.

Table 2.4 Pathogen and indicators present in wastewater and sludges (in numbers)

	Numbers		
Pathogens	Range	Mean	References
E.coli	$3\times10^2 - 6.2\times10^4$	1.5×10^4	[b]Payment *et al.* 2001
	$4.4\times10^5 - 1.1\times 10^6$	-	[a]Pourcher *et al.* 2005
Salmonella	$1.1\times10^1 - 5.9\times10^3$	2.9×10^3	[a]Gibbs *et al.* 1994
	1.2 – 1.3	-	[a]Pourcher *et al.* 2005
	-	6.2×10^2	[a]Dahab and Surampalli 2002
C. perfringens	$3.30 - 4.10\times10^3$	2×10^3	[c]Chauret *et al.* 1999
	$4.5\times10^4 - 8.1\times10^4$	-	[a]Pourcher *et al.* 2005
	$1\times10^1 - 4.5\times10^2$	2.3×10^2	[b]Payment *et al.* 2001

a=g^{-1} dry weight, b= g^{-1} wet weight, c=sludge L^{-1}, ND=not detected.
Adapted from: Sidhu and Toze , (2009)

Table 2.5 Pathogen inactivation (log10) during wastewater treatment processes

Pathogens	AS	MAD	TAD	Comparative indication	References
E.coli	0.9 ± 0.2	1.5 ± 0.6	3.5 ± 0.9	Much lower than E	Gantzer *et al.* 2001
	-	-	6.0	Similar to FC in TAD	Moce-Llivina *et al.* 2003
	-	1.66	-	Similar to Salmonella and Listeria	Horan *et al.* 2004
Salmonella	0.12	0.86-2.26	-	Lower than both FC and FS	Dahab and Surampalli 2002
C.perfringens	0.96	NR	-	Limited, no correlation with bacterial indicators	Chauret *et al.* 1999
	0.34	-	-	Similar to Cryptosporidium	Payment *et al.* 2001

Inactivation data from similar studies were compared for inactivation rates. Aerobic stabilization (AS); Mesophilic anaerobic digestion (MAD) at 35 °C; Thermophilic anaerobic digestion (TAD) at >52 °C; Log reduction=day^{-1}, NR=no reduction, T=Times.
FC= faecal coliforms, FS= faecal streptococci, and E= enterococci.
Source: Sidhu and Toze, (2009)

Clostridium spores are very resistant to water treatment processes and persist longer than *E. coli.* (Horman *et al*., 2004; Payment *et al*., 1993). Consequently, spores of *Clostridium perfringens* are one of the most conservative indicators of fecal pollution and their inactivation during a treatment process positively indicates absence of pathogenic bacteria. However, the value of using *Clostridium perfringens* spores as surrogate indicators for oocysts of protozoan parasites, and enteric viruses is disputed (Hijnen *et al*., 2000; Payment *et al*., 1993). *Clostridium perfringens* spores numbers have been reported to be half those of *E. coli* and fecal coliforms in wastewater, and no reduction in enteric virus numbers was reported even after a 50% reduction in *Clostridium perfringens* spores during the wastewater treatment process(Payment *et al*., 2001).

Pathogens in biosolids amended soil and plants

Most pathogens do not survive in soils or on plants for very long periods of time (Rudolfs, et al. 1950, 1951; Lance, 1977; Akin et al., 1978; Golueke, 1983; Sorber and Moore, 1986). Several environmental factors affect the potential for pathogen survival such as temperature, desiccation, and ultraviolet light, particularly if the pathogens are located near the surface of the soil. Other factors, including pH, organic matter, soil colloidal matter, soil temperature, and competitive or antagonistic organisms, will also negatively impact pathogen survival in soils.

For example the growth of Salmonella and dysentery bacilli was reported to be suppressed in soil by actinomycetes (Bryanskaya, 1966).

Pourcher *et al.* (2007) studied the survival of enteric micro-organisms in sewage sludge following direct land-spreading. The sludge was spread on a sandy loam fine texture soil, at a rate of 80 m3/ha fresh weight. The tested micro-organisms included three of specific sanitary interest: fecal indicators, spores of Clostridium perfringens and enteroviruses. They reported that among the three micro-organisms studied, only Clostridium perfringens was present in relatively high concentrations in the soil (about 102 spores/ g dry matter) confirming the ubiquitous behaviour of this bacterium. Clostridium perfringens, which was present in the soil before the spreading of sludge, was not inactivated during the 2 months of observation. An increase in the concentration of Clostridium perfringens was observed implying some bacterial growth. Concentrations of Clostridium perfringens did not differ significantly from one plot treated with sludge to another.

In most cases, it has been reported that the survival of the micro-organisms is lower in summer and in sandy soils (rather than clay), and when biosolids is spread on the soil surface rather than injected (Nicholson *et al.*, 2005). In addition, the growth of faecal coliforms is favoured by the presence of fertilizers (Estrada et al., 2004).

Guan and Holley (2003) report that *E. coli* O157: H7 and Salmonella survive longer in the soil than Yersinia enterocolitica and Campylobacter intestinalis. In addition, predicting the survival of bacteria is complicated because of the possibility of regrowth (Bastos and Mara, 1995; Gibbs *et al.*, 1997). Because of various environmental parameters influencing the survival of micro-organisms and the complexity of their interaction, results obtained by different workers do not always agree. According to Snowdon et al. (1989) a survival of 12 weeks is frequently observed for bacteria. Gibbs *et al.* (1997) and Jones (1986) found Salmonella in the soil 36 and 37 weeks after spreading biosolids or animal wastes, Watkins and Sleath (1981); Nicholson *et al.* (2005) and Gessel *et al.* (2004) observed the disappearance of these same bacteria in less than 6 weeks.

Sun *et al.* (2006) conducted an incubation experiment to monitor and evaluate the effect of application of four types of sewage sludges (fresh anaerobically digested sewage sludge, air dried sludge, fresh dewatered sewage sludge and air-dried from different waste treatment plants) on changes in numbers of faecal coliforms in soils and hygienic risks over time after sludge application. Soil faecal coliforms were counted after 1, 7, 14, 28, 56 and 84 days of incubation at 25 ± 0.5 ^{0}C and reported that faecal coliforms counts in the biosolids-treated soils decreased substantially with incubation time and were similar to those in the untreated controls after incubation for 56 days.

Air-drying also decreased the quantity of faecal coliforms and reduced the risks following land application of sewage sludge (Sun *et al*., 2005); however, they suggested that land application of air-dried sludges may increase the hygiene risks when re-growth of faecal coliforms occurs. Sun *et al*. (2005) also reported that the sludge-derived faecal coliforms gradually died off after entering the soil and concluded that an appropriate restriction of public access time could be introduced to protect human health.

Pathogens in dewatered biosolids material applied to land will not likely leach out and move through the surface soil to groundwater. The movement of bacteria where dewatered biosolids are applied is markedly different from wastewater. Surface application, including tilling or incorporating biosolids into the upper 15 cm (6 in.) greatly reduces the survival and movement of bacteria (Reddy *et al*., 1981). Sorber and Moore, (1986) reviewed the literature prior to 1986 and concluded that quantitative data describing pathogen survival or transport in biosolids-amended soil were extremely limited.

Biosolids application modifies soil properties, which increases the retention and removal of pathogens. Increased organic matter will lower water percolation and enhance water retention in sandy or gravelly soils. If the application of biosolids increases soil pH possibly through liming, this could affect bacterial survival. The increased organic matter from biosolids' application enhances the indigenous microbial population that could result in pathogen inactivation.

Bryan (1977) reviewed the early literature on the survival of pathogens on crops and found that pathogens do not penetrate into fruits or vegetables unless their skin is broken. Pathogen survival would be very short on fruits and vegetables exposed to sunlight and dry conditions. The survival on subsurface crops such as potatoes and beets would be similar to that in soil (Gerba, 1983). The edible portion of crops that does not come in contact with the soil or biosolids is less apt to be contaminated. Although the time varies, evidence from the literature indicates that the survival rate of pathogens on plants is very short even if the time varies depending upon conditions. Desiccation, temperature, and ultraviolet light are the most important factors in destroying pathogens on plants. Thus, the risk for humans consuming foods where biosolids are land applied is low since most of the biosolids are incorporated into the soil and do not come in contact with edible part of the food crops. According to Epstein (2003) the risk to humans from pathogens in biosolids that are applied to non-edible crops, forestry, and fruit trees is essentially nil.

The application of biosolids on crop land used to produce food crops for human and fodder crops for livestock consumption must not result in unacceptable microbiological or chemical contamination of produce nor adversely impact upon produce quality (EPA, Vic. 2004).

Only treatment grade T1 biosolids need to be used in circumstances where planned cropping strategies will result in direct contact between the soil and human food crops that are potentially consumed raw and these include produces such as carrots, lettuces, strawberries and mushrooms.

Treatment grade T2 biosolids should be used where human food crops that are possibly consumed raw will be protected from direct contact with the soil such as harvested produces greater or equal to one metre above soil surface nevertheless, the biosolids application should not occur within a period of three summer months before expected harvest and during this time windfall should not be collected. Treatment grade T2 biosolids have been subjected to a reasonably intensive pathogen reduction process and thus the combination of treatment with a withholding period and produce separation is considered to provide a proper and realistic level of control (EPA, Vic. 2004).

In Australia, grade T3 biosolids should not be used as part of a planned cropping strategy, although such practice is approved in key jurisdictions such as in US and UK, requiring significant withholding periods of one to three years. However, where land amended with T3 biosolids is subsequently considered for growing food crops that are potentially consumed raw; a default withholding period of three years will be needed, however, grade T3 or better biosolids may be used for crops that will either be cooked at greater than 70°C for two minutes or processed prior to sale to the domestic market. Grade T3 biosolids can be used for crop production that will be cooked after sale to the domestic market, however, the safety of the practice needs to satisfy the relevant government agencies, such as EPA and the Department of Human Services (DHS) (EPA, Vic. 2004).

The natural decay of enteric microorganisms in soil represents an important stage in the multi-barrier approach to protect human health from the residual numbers of potentially infectious pathogens that may be present in sewage sludge recycled to agricultural land (Pike and Carrington 1986).

The risks of pathogens reduction from land applied biosolids in United States and Europe is solely based on the concept of multiple barriers to the prevention of transmission of disease (US EPA, 1993; European Commission, 1986). The barriers include: (i) treatment to reduce pathogen content and vector attraction, (ii) restrictions on crops grown on land to which biosolids have been applied, and (iii) minimum intervals following application and grazing or harvesting.

Successful pathogen risk management requires control to the entire chain of sludge treatment, biosolids handling and application, and post-application activities which may be achieved by devotion to quality management systems based on hazard analysis critical control point principles (Godfreee and Farrell, 2005).

In summary, the literature review describes the digestion, alkali-stabilization, composting, irradiation and pasteurizations processes are frequently used in reducing pathogens, decrease vector attraction to produce a stabilized product that will not putrefy rapidly or produce unpleasant odours. Anaerobic mesophilic (35 - 40 0C) digestion is most widely used to stabilize primary and secondary sludges from municipal wastewaters. However, many types of pathogens can survive such treatments and elevated temperatures are often needed to improve the rate of sludge stabilization and to enhance the reduction of pathogens. The evidence for the correlation between the reduction of indicator bacteria and pathogens during biosolids treatment processes is contradictory.

A number of environmental factors influence the potential for pathogen survival such as temperature, desiccation, and ultraviolet light, particularly if the pathogens are located near the surface of the soil or on a plant. Other factors, including pH, organic matter, soil colloidal matter, soil temperature, and competitive or antagonistic organisms, will also negatively impact pathogen survival in soils. Overall the soil environment is unfriendly and hostile to pathogens. In addition, the survival of pathogens in summer and in sandy soils is lower and when sludge is spread on the soil surface rather than injected.

The leaching and movement of pathogens through the surface soil to groundwater is very low and in most cases are retained in the upper 5 to 15 cm of the soil depth. Surface application, including tilling or incorporating biosolids into the upper 15 cm greatly reduces the survival and movement of bacteria.

Soil properties can be modified by the application of biosolids, which increases the retention and removal of pathogens. Increased organic matter will lower water percolation and enhance water retention in sandy or gravelly soils. The increased organic matter from biosolids' application enhances the indigenous microbial population that could result in pathogen inactivation.

If the edible portion of the crop is not in contact with the biosolids, the likelihood of contamination is low, particularly if the skin of the fruit is unbroken, since pathogen survival is short on fruits and vegetables exposed to sunlight and dry conditions. Thus, the risk for humans consuming foods where biosolids are land applied is low provided that most of the biosolids are incorporated into the soil and do not come in contact with edible food crops.

The health hazards of pathogens from land applied biosolids to non- food crops, forestry, and fruit trees are basically insignificant.

2.6.2. Heavy metals in soil and biosolids

Heavy metals are a group of elements with a relatively high molecular weight (density >5.0 mg/m^3) and, when taken into the body, can accumulate in specific body organs (Ashworth, 1991).

Various agricultural soils may contain considerably higher levels of heavy metals than normally found in natural soils as a result of atmospheric deposition and application of fertilizers, pesticides and biosolids. A number of researchers have reported on significant atmospheric deposition of Pb, Cd, As, Cu and Zn (Haygarth *et al.*, 1995; Hovmand *et al.*, 1983; Berthelsen *et al.*, 1995; Harrison and Chirgawi, 1989). The US EPA has regulated some of the heavy metals in biosolids including As, Cd, Cu, Pb, Hg, Ni, Se, Zn and molybdenum (Mo) (US EPA, 1993).

Biosolids

Biosolids contain heavy metals as a result of atmospheric deposition on land, natural vegetation, food sources (because plant material will contain trace elements), industrial sources, fertilizers and pesticides, human wastes (due to ingestion of food and water) and natural soil. All of these materials can find their way into the sewage system and eventually end up in the wastewater treatment plant and into biosolids (Epstein, 2003).

The addition of chemicals such as lime and ferric chloride in biosolids during the treatment processing can affect pH, composition, and chemical species. These chemical species could induce solubility and hence mobility in the soil or uptake by plants. Thus the biosolids processing and stabilization method also affects heavy metal characteristics and behaviour **(**Richards *et al*., 1997; Green *et al*., 2003 and Fuentes *et al*., 2004).

Conventional fertilizer

Heavy metals are ubiquitous being found in natural soils and plants. They are also in fertilizers since they are part of the mineralogical composition of the mined materials. This is especially true of many phosphate fertilizers that can contain high levels of Cd and Zn (Epstein, 2002; Mermut *et al*., 1996). Lee and Keeny (1975) estimated that 2150 kg of Cd is added annually to Wisconsin soils through fertilizers and biosolids, with much of it coming from fertilizers than biosolids.

Raven and Loeppert (1997) analysed 16 fertilizer materials. Three were primarily nitrogen (ammonium) products, eight were phosphate materials, and four were potassium sources. They also analysed sewage sludge, organic materials and liming materials. Among the fertilizers, phosphate sources had the highest concentration of heavy metals. Potassium and nitrogen fertilizers had insignificant amounts. Cadmium in phosphate fertilizers ranged from 0.7 to 48.8 mg/g; Cu from 0.68 to 19.6 mg/g; Ni from 0.6 to 50.4 mg/g; Pb from < 0.2 to 29.2 mg/g, and Zn from not detected to 33.5 mg/g. They concluded that heavy metal concentrations generally decreased in this order rock phosphate >biosolids > commercial phosphate fertilizer > organic amendments and liming material > commercial K fertilizers > commercial N fertilizers.

Factors affecting heavy metals solubility in soil and biosolids

pH and EC

Aspects of soil that mainly affect heavy metals uptake include soil pH which is one of the main variables impacting upon metal solubility. The equilibrium between adsorbed and dissolved forms regulates the concentrations of heavy metals in the soil solution. All heavy metals with the exception of Mo and Se, are more soluble and mobile at low pH. The solubility of metal carbonates, phosphates and sulfides is increased at low pH (Lindsey, 1979). The bioavailability of heavy metals to plants, in most cases is enhanced below pH of 6.5 (Chaney, 1973). Approximately, a 100-fold decline in activity (effective concentration) of Zn and Cu occurs as soil pH increases by one unit. A decrease in soil pH not only raises their bioavailability but also increases their mobility through the soil profile. Anderson and Christensen (1988) stated the pH is more important than any other soil property in controlling Zn mobility.

A consequence soil pH decline usually occurs after sludge application increasing metal solubility and mobility (Hinesly *et al*., 1972; Boswell, 1975; Robertson *et al*., 1982; Qureshi *et al*., 2003). Richard *et al*. (2000) reported that soil acidification was the result of the mineralization of sludge-derived organic N and S. Changes in the soil environment after biosolids application determines the rate of release of sludge added heavy metals which depend on the nature of the sludge and soil types (McBride, 1998; Richards *et al*. 1997, 2000).

Kim *et al*. (2007) conducted a greenhouse study using silt loam and fine sandy loam soil amended with alkaline stabilized sludge, dewatered-digested sludge and composted sludge at elevated heavy metal loadings and reported elevated levels of Cu and Zn in the $CaCl_2$ extracts in the early cropping seasons resulting from the low soil pH due to the most recent sludge application. Regardless of soil type, the lowest soil pH was achieved by the dewatered sludge treatments indicating that dewatered sludge had more acidifying effect than the composted biosolids, since, during composting some of the N and S was already mineralized, the compost had lower effect in reducing soil pH than dewatered sludge.

Regardless of the soil type, the alkaline stabilized sludge products increased the soil pH and reached a constant pH with time. This condition decreased Zn extractability, although such increases in soil pH did not reduce the solubility of Cu in the sludge through time. Over all, they concluded that sludge amendment had a considerable effect in changing soil pH and hence the extractability of Cu and Zn.

Stamatiadis *et al*. (1999) assessed the effect of compost and fertilizer application on silty clay loam soil quality in a field experiment. Compost was applied at rates of 0, 22 and 44 t/ha which were split to include fertilizer 165 kg (NH_4NO_3)-N/ha and no fertilizer plots. It was reported that a high soil EC in plots receiving 44 t/ha of compost in the first 0-20 cm of soil which probably resulted from a high compost salt content. They also noted a short term stabilization of soil pH and a decrease in water infiltration rates due to compost application. The pH stabilization prevented acidification due to fertilizer application. They concluded that frequent application of composts with high EC values may bring about depletion of N, reduced nutrient cycling and impaired crop growth probably due to increased soil salinity.

Ulrich (1987) also observed higher cation exchange capacity and base saturation, in soil treated with compost as compared to soil without compost, and reported no correlation between pH to NO_3-N and concluded that composts had no acidifying effect.

The availability of heavy metals in composted biosolids and sewage sludge amended soil is controlled by soil pH; decrease in soil pH increases the mobility of the most labile elements in soil particularly Zn, Ni and Cd (Smith, 1994a, b). Compost or biosolids additions in short to medium term may alter the soil pH which could impact metal availability and potential transfer to crops (Smith, 2009). The soil pH value has an important role in controlling metal availability. In most cases, metal extractability and uptake decreases with increasing soil pH value; compost application usually increases soil pH, thus reducing crop uptake, (Smith, 2009).

Compared to the other substrates, MSW- compost has reasonably high electrical conductivity due to increased salt contents (Maftoun *et al.*, 2004) and could reduce the overall bioavailabilities of metals due to sorption processes and thus provide an effective soil remediation technique (Brown *et al.*, 2003, 2004).

Organic matter and CEC

The complexion and binding of heavy metals in the organic matter reduces their availability to plants. The high CEC of organic matter enhances its metal binding capacity; this behaviour of organic matter significantly contributes to the overall CEC of a soil. Sandy soils usually have low CEC than clay soils (Epstein, 2003). Understanding the role of CEC of soil on heavy metals availability helps in making recommendations regarding the application of heavy metals additions via biosolids to soils based on the CEC of a given soil (USEPA, 1980).

Reactions between elements

Availability of heavy metals to plants and their mobility through the soil system depends on interactions with other elements. The hydrous oxides of Fe and Mn can interact with some of the heavy metals through sorption and desorption mechanisms and hence reduce their availability to plants (Jenne, 1968; Quirk and Posner, 1975). Phosphorus also combines with metal ions to form soluble or insoluble complexes (Epstein and Chaney, 1978). Barrow,

(1987) showed that orthophosphates could either increase or decrease Zn retention in soil depending on pH. McBride (1985) reported a decrease in sorption of Cu in soils when orthophosphates were present.

Total and available forms of heavy metals

Total heavy metals

Total heavy metal content is not a good predictor of bioavailability and potential risk assessment; however, it can be used as a valuable indicator of soil deficiency or contamination (Alva *et al*., 2000; McLaughlin *et al*., 2000). Due to diverse and complex distribution patterns of heavy metals among different chemical species (Chen *et al*., 1996), the total metal concentration alone does not give sufficient information on risk of bioavailability and toxicity and metals capacity to remobilize in the environment (Knight *et al*., 1998; Chaudri *et al*., 2000; Su and Wong, 2003; Wei and Liu, 2005). A genuine assessment of the environmental hazard associated with elevated soil heavy metals will depend on the amounts of easily mobile and bioavailable forms (Weber *et al*., 2007).

Bioavailable forms of heavy metals

The major objective of micronutrient (metals) soil tests is to separate nutrient deficient from non-deficient soils, and to indicate when a profitable response to application of specific micronutrients might be expected. Micronutrient soil tests are also used to indicate possible nutrient and heavy metals toxicities. Hence, extraction solutions containing chelating agents such as DTPA (diethylenetriaminepentaaceticacid) remove micronutrient cations adsorbed in the solid phase together with water-soluble constituents. In this regard they may simulate the action of plant roots. Consequently, extraction with DTPA has been used to assess micronutrient fertility of soils (Lindsay and Norvell, 1969a, 1978).

The DTPA extraction solutions also extracts the exchangeable, organically complexed and carbonate forms of heavy metals in soil and this extraction gives valuable information on metal availability and has the tendency to correlate with plant uptake of heavy metals (Bidwell and Dowdy, 1987; Sommers *et al*., 1991; Hooda and Alloway, 1994). Metals that are soluble, exchangeable and loosely adsorbed are reasonably labile and bioavailable (Kabata-Pendias and Pendius, 1992; Alva *et al*., 2000; McLaughlin *et al*., 2000). McGrath

(1984) reported that once biosolids applications cease, Ethylene diamine tetra acetic acid (EDTA) extractable metals also decrease with time suggesting that metals may revert to less available forms through time

For quick evaluation of the phytoavailability of heavy metals, unbuffered extraction solutions such as $CaCl_2$, NH_4Cl can be used (Beckett, 1989); however, there are cases where such salt solutions do not indicate the plant available fraction of heavy metals, hence the use of other extractants such as DTPA and hydroxylamine are better alternatives (Gupta and Aten, 1993).

Table 2.6 summarizes the effect of incorporation or surface applications of various biosolids and sludge types on the levels of total and DTPA extractable heavy metals in amended soil.

Increases in total Cu, Zn, Pb and Cr due to various biosolids applications under field conditions were reported by Walter ***et al***. (2002). A similar increase in total Cu, Zn, Pb and Cr levels using pot experiments were observed by Sims and Kline (1991) and Zheljazkov and Warman (2004).

Table 2.6 The effect of various organic amendments on total and extractable heavy metals in amended soil under field and glass house conditions

Organic waste types	Experimental details	Soil	Crop types	Effect on heavy metals accumulations in soil	References
anaerobically digested biosolids	40, 80 and 120 t/ha surface applied under field condition	S	herbaceous mixture of plants	Total soil Zn, Pb, Cd, Ni, Cr and Cu increased however DTPA extractable heavy metals did not change	Walter *et al.* (2000)
Anaerobically digested biosolids	50 and 100 t/ha incorporated under field condition	T	winter wheat	Total and DTPA extractable heavy metals increased, but not Ni in the low biosolids treatments.	Walter *et al.* (2002)
Co-composted sewage sludge	0, 11, 22, and 44 t/ha), limed to four pH values (pH 5.3–7.2) incorporated under glass house condition	U	winter wheat and soybean	Increases in total soil Cd, Cr, Cu, Ni, Pb, and Zn, at the highest rate (44 t/ha). It had little effect on Cd, Cr, or Pb in either crop, but consistently increased Cu, Ni, and Zn in vegetative tissues of wheat and soybean, and Ni and Zn in soybean grain. Liming decreased Mn and Zn in wheat and soybean, and Ni in soybean grain, but rarely affected plant Cd, Cr, Cu, or Pb.	Sims and Kline (1991)
Activated sludge and facultative stabilized pond sludge	100 t/ha incorporated under glass house condition	F,H,I	sorghum bicolor	Increased DTPA extractable, Zn showed the highest increase from 1 to 25 times the control plot	Mendoza *et al*. (2006)
Municipal solid waste and municipal solid waste compost	21, 41 and 62 t/ha MSW and 60 t/ha MSWC incorporated under field condition	I	No crop	Cu, Zn and Pb remained to the upper 5 cm soil layer. The low water extractable fraction of these metals in MSW and MSW-biosolids compost was a major factor limiting the transport of these metals to lower soil horizons.	Breslin (1999)
Municipal solid waste compost and cow dung manure	60 kg N per ha MSWC, 60 kg N per/ha cow dung manure with 30kg N/ha urea as supplement	F	rice	Increased DTPA soil Cu and Zn over cow dung manure	Bhattacharyya *et al*. (2006)
Municipal solid waste compost	First, 175, 350, and 525g/pot, second 125, 250, and 375 g/pot incorporated under glass house condition	I	Swiss chard, Basil , Dill , and peppermint	Increased the concentration of Cu and Zn in all fractions, increased Mn in acid extractable (ACID), iron and manganese oxides (FeMnOX), and organic matter (OM) fractions, but decreased slightly exchangeable-Mn. reduced bioavailability and transfer factors for Cu and Zn	Zheljazkov and Warman (2004)

Table 2.6 continued The effect of various organic amendments under field and glass house conditions on total and extractable heavy metals in amended soil.

Aerobically-digested sewage sludge (AES) and anaerobic lagoon septic waste (ANS), a sewage sludge compost	30.6 t/ha compost and 10.7 t/ha of ANS to the forage and 25.7 dry t/ha compost and 6.5 dry t/ha aerobically-digested sewage sludge to the corn. Surface applied under field condition	G	grass forage	Cu in aerobically-digested sewage sludge amended corn stover was greater than the compost and fertilizer-amended corn in yr 1 but was not different in yr 2 possibly due to high replicate variability. Applications of both organic wastes increased zinc in the grass forage tissue but the increase was greater from the ANS, which they related with its total Zn content.	Warman and Termeer (2005)
Aerobically digested biosolids	At 0, 42, 84, 126, 168, and 210 t/ha incorporated under field condition.	F	raddish and romaine lettuce	Linear increase in DTPA Cu and Zn but a decrease in metal availability with time.	Evanylo *et al.* (2006)
Composted and non-composted sewage sludge	30 and 50 g/kg/ pot incorporated under glass house condition	F,V	with out crop	Increase in available Fe, Cu, Mn and Zn concentrations. Continued increase in the available Fe, Cu, Mn and Zn throughout the incubation period.	Moral et al. (2002)
MSW-compost, sewage sludge compost and MSW co composted with sludge. Two soil pH treatments (5.8 & 6.5)	0, 25, 50 and 100 x 10(6) g/ha incorporated under field condition	W	tobacco	DTPA-extractable Zn, Pb, Cd and Cu increased with increasing soil pH at the 100 x 10(6) g/ ha rates of co composted in contrast to the usual response to increasing soil pH, which generally reduces the availability of heavy metals in soil.	Baldwin and Shelton (1999)
Spent mushroom compost ,forced aeration compost and inorganic fertilizer	0, 25, 50 and 100 t/ha incorporated under field condition	-	barley	Increase in soil pH, EC and DTPA extractable Zn, but a decrease in DTPA extractable Fe and Mn. Increases in available K, Ca, Mg and Na.	Courtney and Mullen (2007)
Coal fly ash-stabilized sludge	0%, 5%, 10%, 35% and 50% proportions incorporated under glass house condition	H	corn	Increase in pH caused precipitation of soluble cations in the ash sludge mixture, resulting in reduction in both EC and in DTPA Cu, Zn, Ni and Cd following ash amendment	Su and Wong (2003)

The superscripts loamy clay (F), silty loam (G), fine loam (H), sandy loam (I), Lithic xerothents (S), Haploxeralf caliorthid soil (T), coastal plain soil (U), sandy clay loam (V) and clay soil (W) attached on soil, refers to various soil types respectively.

Moral *et al*. (2002) found increases in DTPA extractable Cu, Zn, Mn and Fe using composted and non-composted sewage sludges under glass house conditions. Increases in DTPA extractable Cu and Zn due to applications of municipal solid waste compost were also reported by (Bhattacharyya *et al*. 2006) under field conditions; and significant increases in Zn due to applications of activated sludge in a pot experiment was reported by Mendoza *et al*. (2006).

A decrease in DTPA extractable Cu, Zn, and Ni in ash stabilized sludge were reported by Su and Wong (2003) and a reduction in DTPA extractable Fe and Mn following application of spent mushroom compost were also noted by Courtney and Mullen (2007). However, Walter *et al*. (2000) reported no change in DTPA extractable heavy metals after the applications of anaerobically digested biosolids in a field experiment.

Baldwin and Shelton (1999) also reported increases in DTPA-extractable Zn, Pb, Cd and Cu with increasing soil pH after the applications of co-composted biosolids in contrast to the usual response to increasing soil pH, which frequently reduces the availability of heavy metals in soil.

Mobility of heavy metals

The heavy metal chemical form and its association with inorganic and organic soil constituents to a great extent affect its possible movement, retention and plant uptake (Lake *et al*., 1984). Heavy metals in soil exist in a range of physicochemical forms which include "free or complexed ions in soil solution, adsorbed species on the surface of clays, complexes with Fe and Mn oxyhydroxides, or organic matter, contaminants in the lattice of secondary minerals such as phosphates, sulfides, or carbonates, and in the crystal lattices of primary minerals" (Berti and Jacobs, 1996). Most of these physicochemical forms involve complexation of the metal (Sposito *et al*., 1982; O'Conner, 1988). For trace metals to move in the soil or be taken up by plants, metals have to be in the soil solution.

Richard *et al*. (1997) examined the leachability of trace elements as indicated by the toxic characteristic leaching procedure of dewatered, composted, N-Viro, pellets, and incinerator ash. Toxic characteristic leaching procedure, developed by US EPA (1987) is usually used to indicate potential leachability of metals. It was reported that, with the exception of N-Viro, very small percentages of Cd, Cr, Cu, Mo, Ni, Pb, and Zn were extracted as a percentage of total content which indicated the potential for leaching and mobility in the soil system is extremely low.

Berti and Jacobs (1996) stated that no fractionation method is completely effective in dissolving each distinct form of a heavy metal. It was showed that the toxicity characteristic leaching procedure, used to illustrate leaching of heavy metals from wastes deposited in landfills, is not sufficient for determining the bioavailability in soils. Baveye *et al*. (1999) reported that metal extractions using nitric acid and DTPA dissolve only a portion of the total metal in soils.

Dowdy and Volk (1983) reported from their extensive review of the literature the conditions where movement and leaching of biosolids-borne heavy metals could occur. They found very little evidence of heavy metal movement beyond the zone of biosolids incorporation. Several studies (Chang *et al*. 1984; Williams *et al*. 1987) also reported that there was very little movement of heavy metals into the subasoil or below 30 cm. Dowdy *et al*. (1991) found that Cd and Zn concentrations were higher in the 0.32 to 0.62 m depth compared to the control. Zn concentrations increased with biosolids application but this was not the case for Cu. Low amounts of biosolids-borne Cd and Zn were found in the subsoil after a 14-year period of substantial biosolids application than the control plots.

In contrast**,** Antoniadis and Alloway (2003) investigated the movement of heavy metals by taking soil samples from a heavily sludged area from a farm treated with 8 t/ha sludge and observed that the movement of heavy metals in the heavily sludged area was significant moving down to 80 cm soil depth compared to the control. In the sludge treated farm, the concentrations of Pb and Zn and the organic matter content were higher than the control down to 20 cm, and there was a considerable leaching of Ni down to 80 cm soil depth. They suggested the possibility that the metals bound onto organic surfaces moved along with organic matter down to that depth. Lower pH and soluble organic matter of soil that is induced by sludge application often promote dissolution and assist movement of metals (Antoniadis and Alloway, 2002; Richards *et al*., 2000). Sabien *et al*. (2008) conducted a field experiment using sewage sludge compost and showed that more heavy metals were found as mobile species in sandy soil than in clay soil. They observed increases in Cu levels due to the effect of sewage sludge compost of anthropogenic origin with lower pH and high organic matter content; however, the mobility of Cu was hindered to nearly the initial level after 3-4 weeks, suggesting that soils have a high specific adsorption capacity to immobilize Cu of anthropogenic origin. Hence, the effect on heavy metal mobility and potential availability was greater in sandy sewage sludge compost-amended soils than in clay soils and increased with an increasing amount of sewage sludge compost.

Factors such as receiving soil properties (pH, EC, clay content, hydrous metal oxide content) and management practice influence metal bioavailability (Mahler *et al*., 1987; Hooda and Alloway, 1993b; Richards *et al*., 2001). With regard to heavy metals availability in sewage sludge, there are two apparently contradicting theories in the literature. The first theory suggests that often the inorganic fraction of sewage sludge is responsible for the strong binding effect of heavy metals. Carbonate, phosphate and sulphide form insoluble solid phases with many metals and have been observed to account for the significant portion of several metals in sludges (Karapanagiotis *et al*., 1991). Non-crystalline Fe and Al oxides are considered for the principal role in metal retention in sludges. The sludge inorganic fraction is primarily responsible for heavy metal retention (Chaney and Ryan, 1993; Brown *et al*., 1998; Li *et al*., 2001) and that with time sludge-borne metals will become less available.

Hence, it was suggested that sewage sludge properties dominate metal chemistry and bioavailability in the short to medium term, in the zone of sludge incorporation (Lake *et al*., 1984; Zufiaurre *et al*., 1998).With time, it is thought that the sludge properties will have progressively less influence over metal behaviour and that soil characteristics ultimately control metal speciation, irrespective of the speciation in the original sludge (Smith, 1996). The time frame over which this occurs is thought to vary greatly; from less than 12 weeks to over 8 years (Parkpain *et al*., 1998; Smith, 1996).The factors that govern this temporal change have not yet been identified.

On the contrary, the second theory proposes that metals held in humic substances, biomass and noncrystalline materials that are presumably to be released during ageing and organic matter decomposition (Baldwin *et al*., 1983; Martinez *et al*., 2001). Due to the decomposition of organic matter, the composition of sludge may be altered which tends for heavy metals previously bound by organic matter fractions to be released to the soil solutions. As organic binding sites are lost through microbial degradation of the organic matter, sludge borne metals become increasingly available (Hooda and Alloway 1994), because of this reasons the degradation of the organic matter contained in sludge is considered by some to be an important factor in the release of heavy metals in sewage sludge-amended soils (McBride, 1995; Sadovnikova *et al*., 1996). Indeed for some heavy metals this has been demonstrated soon following sewage sludge applications (Richards *et al*., 2000). Certainly some of the soluble complexing organic ligands in the sludge are responsible for elevating the mobility of some elements, especially Cu in soils. In this regard, McBride *et al*. (1999) indicated that the solid–solution partition coefficient (*K*d) was actually reduced from 221 to 208 (l/kg) for Cu when sludge rates were increased from 20 to 50% of a soil mix by weight. Organic matter in sludges may bind metals, but this may be offset by the formation of soluble organo-metal complexes and the loss of complexation capacity through organic matter degradation (Karapanagiotis *et al*., 1991).

Merrington and McLaughlin (2003) reviewed the available literature on the influence of sewage sludge properties on sludge-borne metal availability, and indicated that sludge properties have a significant influence on metal availability and the potential for metal release from sludges mainly depends upon sludge compositional factors.

There is conflicting evidence in the literature as to whether inorganic or organic fractions of sludge play the dominant role in controlling metal availability. Evidence exists for both theories, and it is likely that sewage sludge compositional (both inorganic and organic) and metal factors have a significant role in affecting sludge-borne metal release properties, however it is not clearly understood, and hence suggested greater use of spectroscopic techniques that provide quantitative information on sludge organic matter chemistry (Merrington and Mclaughlin 2003).

It was also suggested that sewage sludge and compost applications to agricultural and other non-metal contaminated soils elevate the soil content and the availability of heavy metals to be taken up by crop plants. The availability in the soil depends on various factors, and these include the nature of the chemical association between a metal with the organic residual and soil matrix, the pH value of the soil, the concentration of the element in the compost and the soil, and the ability of the plant to regulate uptake of a particular element (Smith, 2009).

In summary, different agricultural soils may well contain noticeably higher levels of heavy metals than usually found in natural soils due to application of fertilizers, pesticides, biosolids and atmospheric deposition.

Undoubtedly heavy metals exist in soil in various physicochemical forms. The mobility, retention and plant uptake of heavy metals largely depends on the chemical forms and its relationship with organic and inorganic soil constituents.

The decomposition of soil organic matter by microorganisms could produce organic acids, together with carbon dioxide and water, which tends to form carbonic acid. This lowers the soil pH increasing the solubility of heavy metals subsequently making them more available to plants. In contrast, an increase in soil pH due to compost applications was also reported by a number of researchers.

The availability of heavy metals to plants and their mobility through the soil system also depends on interactions with other elements. Some components such as organic matter can interact with certain heavy metals to reduce their availability to plants.

In general, after the application of biosolids, heavy metals are more likely to become bioavailable when the biosolids are sourced from waste water treatment plants (aerobically and anaerobicaly digested biosolids) and composted biosolids than municipal solid waste composts. Several researchers have indicated that heavy metal mobility and potential availability was greater in soils which are sandy or have had their pH lowered by the application of biosolids than in clay soils.

2.6.3. Plant uptake of heavy metals from biosolids

The transfer of heavy metals into plants

There exists a high tendency for metals (Cd, Cu, Zn) to be transferred from biosolids to soil and consequently to crops and to the environment. The concern is the long-term influence of these metals at elevated concentrations in the environment while they continue to stay in the soil for a long period (McGrath, 1987). As the result of heavy metals adsorption on hydrous oxides, clays, and organic matter, the formation of insoluble salts, or the presence of residual biosolids particles, metals from land applied biosolids stay in the zone of biosolids incorporation usually (0-15 cm depth) (Alloway and Jackson, 1991).

Chaudri *et al*. (2001) indicated that uptake of Cd by wheat was strongly influenced by soil pore water, while Bingham *et al*. (1975) showed that Cd content of plants varies according to plant species and plant tissue where cereals and legumes accumulated less Cd in shoots than leafy vegetables.

After the cessation of long-term biosolids application, the continued mineralization of biosolids organic matter may release heavy metals into the soil, however the significant proportion in the availability of metals from biosolids to plants is in the period soon after biosolids application, nevertheless as the decomposition of organic matter declines through time, metal availability could be decreased (Bidwell and Dowdy, 1987).

Heavy metals uptake by plants might not only be influenced by their concentrations in soil, their chemical forms, physicochemical properties of the soil, but also by plant nutritional status, stage of growth, and other related factors (Adriano, 1986; Chlopecka, 1996a,b).

In sandy loam and loam soils, activated sludge or facultative stabilization pond sludge application increased the concentration of foliar Cu by 46–87% compared to the control plot (Mendoza *et al*., 2006). Gregory *et al*. (2006) also reported higher Cu and Zn concentrations in the corn grain and stover (corn stalk), radish globes and tops, and lettuce in the biosolids treatments than in the control plots and increased with biosolids rate as pH declined. Cu and Zn accumulations in particular were dependent on the plant species, as has been reported (Corey *et al*., 1987; Berti and Jacobs, 1996).

Increased content of bioavailable Cu in soils and increased Cu content in plant tissue following compost application were also reported (Murillo *et al*., 1997; Ozores-Hampton *et al*., 1997).

Nogales *et al*. (2001) conducted a column study in a greenhouse to determine the availability, extractability and leachability of metals in a degraded, non-calcareous soil amended with different biosolids (200 t dry solids/ha). The biosolids investigated were dewatered, anaerobically digested biosolids, composted biosolids and biosolids-ash. The columns (26 cm) were planted with wheat (*Triticum aestivum* L. cv Mexa). They reported that Cu and Zn uptake by wheat plants increased due to biosolids applications, although to different degrees. The highest concentrations of Cu and Zn in the different plant parts were caused by the application of digested biosolids, followed by the composted biosolids and biosolids-ash. However, they did not detect Ni, Co, Pb, Cr and Cd in the different plant samples analysed. The application of the different biosolids increased the total concentrations of Cu, Zn, Pb and Cr, measured after harvest, in the surface layer of the soil to similar levels, but it did not cause measurable changes in the total soil concentrations of Co and Ni.

Metals derived from sludge are in most cases bound to the organic matter, sulphates, carbonates and are less bioavailable to plants than the more soluble forms found in commercial fertilizer, nonetheless plant uptake of heavy metals depends on the nature of the metal, physicochemical properties of the sludge/soil mixture and plant species. Due to the decomposition of biosolids organic matter, the application of heavy metal contaminated biosolids may increase metal bioavailability or change the soil metal fractions through time

(McBride, 2003**).** Likewise other researchers (Lane, 1988; Gaskin *et al*. 2003) also reported elevated levels of heavy metals in biosolids amended plots.

To assess the potential impact of long-term sewage sludge application on soil health, Mc Bride *et al*. (2004) conducted a greenhouse experiment using a soil column with dewatered, composted and pelletized sludge products. They found that Mn concentrations in clover shoots grown on a number of the soil columns were very high and seemingly phytotoxic. This result was due in large part to depressed soil pH; those soil treatments which produced strongly acidified soils typically produced very high clover Mn concentrations, at times exceeding 1000 mg/kg. Soil pH had a strong influence on the level of extractable metals, and since the tested sludge products affected pH differently, it was essential to consider pH in the comparison of different sludge treatments with controls. Shober *et al*. (2007) analysed biosolids amended and unamended soil and plant samples taken from production farm in Pennsylvania and observed extremely low tissue concentration of Pb indicating minimal uptake and translocation of Pb into the crop tissue. They also reported that crop tissue Ni concentrations were not correlated with Ni in any soil fraction; likewise, concentrations of Cu and Zn in soybean, alfalfa, and corn grain tissue were not correlated with Cu or Zn in any soil fraction; however, concentrations of Cu in all soil fractions were significantly correlated with concentrations of Cu in orchard grass tissue. Concentrations of exchangeable and reducible Zn were also significantly correlated with Zn in Sudan grass tissue.

Mechanism, rate and extent of plant uptake

The adsorption of heavy metals on mineral surfaces is probably the dominant process controlling metal solution activities. Activity is the effective concentration. When metals are added to the soil through the application of biosolids, the solution metal activities would be controlled by adsorption to biosolids and soil mineral surfaces, provided that the amount of metal added did not exceed the capacity of specific surface adsorption sites in the soil (Corey *et al*., 1981).

Epstein and Chaney (1978) have suggested that the metal chemistry of a particular biosolids may be important in the availability of the metal to plants. Heavy metals can also revert with time to chemical forms less available to plants**.**

Sposito *et al*. (1983) found that eventually Zn from biosolids reverted to the less available carbonate. However, metal reversion in soil is small and/or slow and can be reversed by soil acidity (Logan and Chaney, 1983). The form of the heavy metal in soil affects its solubility and, therefore, the potential for movement through the soil and uptake by plants.

Organic matter is the most important chelating agent. Once the trace element enters the root system, at least two phases involve movement and accumulation in upper plant tissues (Chaney, 1975). The first phase involves movement to and release into the xylem sap while the second involves movement in the xylem sap to plant tissues.

Chaney (1983) states that a soil-plant-barrier protects the food chain from toxicity of a trace element when one or more of the following processes limits maximum levels of that element in edible plant tissues to levels safe for animals. Insolubility of the element in soil prevents uptake and mobility of an element in fibrous roots and prevents translocation to edible plant tissues. Phytotoxicity of the element occurs at concentrations where the element, in edible plant tissues, is below a level that is injurious to animals but high enough to cause injury or death to the plant. In biosolids, the elements most likely to be phytotoxic include Cu, Ni and Zn. Cu and Ni toxicity retards growth and can inhibit Fe translocation, resulting in Fe deficiency and chlorosis. Zn toxicity can also result in retarded growth and symptoms similar to Fe deficiency. Phytotoxicity is also dependent upon soil pH, crop species and cultivars, biosolids' metal concentration and other soil and climatic factors (Chaney, 1994).

At higher cumulative heavy metals biosolids loadings, enrichment of more labile soil fractions led to increased crop uptake of Cu and Zn from biosolids-amended soils.

Hence, it was concluded that several trace elements may be taken up and accumulate in the root system with only limited translocation into aboveground tissues**.**

Studies of wheat root and shoot tissues in biosolids amended soils reported substantially larger amounts of Pb, Cu, and Ni in the roots of wheat (***Triticum spp***.) than in shoots (Qian ***et al***., 1996).

Adriano (1986) suggested that most of the metal retained in the roots is present in the soluble form in vacuoles of root cells, and more specifically in the protoplasmic fractions of the roots. One possible reason was that the self-adjusting mechanisms of plants play an important role on sequestering the metal in their roots. Only small amounts of metal are translocated to the above ground parts of plants. A large part of the Cu and Zn content in roots may be retained in root cell walls (Brun ***et al***., 2001).

Several long-term studies on plant uptake of metals from sewage sludge/biosolids revealed that plant uptake is a function of plant species, individual heavy metals, soil characteristics and sludge/biosolids characteristics. Sludge/biosolids is both a source and a sink for trace elements. Heavy metal uptake by plants may follow many different rate response functions such as linear, sympatric, no response, or even negative (Page *et al.*, 1987).

Several researchers reported that the uptake by various crops was not linear with trace elements or sludge/biosolids' application rate, but rather approached a maximum and then levelled off or decreased **(**Logan and Chaney, 1983; Corey *et al.*, 1987; Chaney and Ryan, 1992). Such an incident was called a plateau response. For low-metal biosolids, the phytoavailability is controlled by the biosolids' chemistry (Brown *et al*., 1998).

Beckett *et al*. (1979) suggested that the organic matter in biosolids is responsible for the binding effect of metals. McBride (1995) asserted that as the organic matter decomposed, heavy metals will be released into more soluble forms and result in increased uptake from biosolids.

Chang *et al*. (1997) indicated that the "sludge time bomb hypothesis" may show a plateau response during the course of biosolids' application. McBride (1995) stated, "Because soils have a finite capacity to immobilize metal by adsorption or precipitation reactions, without the protective effect of the sorptive material in the sludge itself, a Langmuir-type relationship would be expected." The plateau effect implies that biosolids are both a source and a sink for trace elements applied to soil. At low biosolids' application, the soil binds the elements and plant uptake is linear.

Similarly, for soils with a low level of metal contamination, a linear correlation between the concentration of Cd and Zn in a given soil and the Cd and Zn concentration in plants is frequently observed (Iyengar *et al.*, 1981; King, 1988; Zhao *et al.*, 1997).

In contrast, at very high biosolids' application rates, the biosolids matrix affects the binding of trace elements. The plateau theory, therefore, speculates that the concentrations of heavy metals in plant tissue will reach a plateau as biosolids mass loadings are increased and they will remain at a plateau after the termination of biosolids' application. Chang *et al*. (1997) used a set of experimental data obtained from a field biosolids' land application to evaluate the hypotheses of the plateau and the time bomb and indicated that with those set of data, an actual plateau or time bomb was not evident. Biosolids' application had reached 2880 t dry solids/ha, which probably represented a worst-case scenario in terms of pollutant loading.

Sloan *et al*. (1998) evaluated the recovery of biosolids-applied heavy metals in a field experiment. The results of the study showed that biosolids organic matter decomposes slowly when applied to a well-rained silt loam in a temperate climate. Therefore, the rapid release of biosolids-derived heavy metals is unlikely to occur.

Bidwell and Dowdy (1987) showed that Cd and Zn availability to corn, following termination of land application of sewage sludge, decreased with time. Corn was sampled for 6 years after termination of three annual applications of sewage sludge. Cumulative sludge applications totalled 0, 60, 120 and 180 t/ha. The results showed that Cd and Zn concentrations in corn stover and grain increased with sludge applications where high levels of the metals are applied to the soil. There was a decrease in the concentration of Cd and Zn in both the corn stover and grain, with time, following application of sewage sludge. The corn grain contained significantly lower concentrations of the metals than the stover. For most of the treatments and time, there was no difference in Cd concentration in the grain.

Logan (1997) conducted a field study from 1991 to 1995 reported that different trace elements behaved differently following biosolids' application. Cd, Cu and Zn concentrations in corn increased significantly with biosolids' application, while Ni and Pb levels were lower than the control. Cd, Cu and Zn concentrations in corn exhibited a plateau-type response. Lettuce concentrations increased linearly with biosolids' application for Cd, Cu and Zn in all years; linear regression slopes generally declined and stabilized after the first 2 years.

Likewise, Street *et al*. (1978) also found that the rate of Cd uptake by corn seedlings plateaued with respect to increasing concentrations of Cd in soil amended with sludge. Dowdy *et al*. (1978) reported that the concentration of Zn and Cu in bean leaf tissue increased with amount of metals supplied to soil through additions of sludge, until they reached a maximum concentration which did not respond to further applications of metals.

The idea of plateau hypothesis was discussed by Chaney and Ryan (1993) in which the concentration of metal in plants approaches a plateau with increasing sludge application rate because addition of the sludge to the soil increases not only the total soil metal content, but also the metal adsorption capacity of the soil, thereby potentially reducing total metal availability.

Precipitation reactions could limit metal solubility (Christensen and Tjell, 1984; Mahler *et al*., 1978). Increases in the total metal concentration in the soil above a critical limit for metal precipitation would not lead to further increases in metal concentrations in the soil solution, and there would therefore be no further increase in absorption of metals by the plant roots.

The uptake of Zn and other transition metals by plant roots is suggested to occur through a channel **(**Guerinot and Eide, 1999; Van der Zaal *et al*., 1999) or carrier mediated process which becomes saturated at relatively low concentrations of substrate in solution (Chaudhry and Loneragan, 1972; Hamon, 1995; Grotz *et al*., 1998;). At metal concentrations at which the uptake mechanism is saturated, there would be no further increase in metal uptake with increasing concentrations in the soil solution.

However, McBride (1995) proposed that plant and not soil mechanisms could account for the observed plateau in metal uptake. Baker (1981) described different strategies which could allow plants to tolerate elevated concentrations of metals in the soil. One strategy was the so-called 'excluder' mechanism whereby the plant blocks the translocation of metals from the root to the shoot in order to reduce the accumulation of toxic metals in the leaves.

To examine concentrations of Cd and Zn in the plants and in corresponding rhizosphere soil solution, Hamon *et al*. (1999) conducted a greenhouse incubation experiment using *Raphanus sativus* L in a soil historically amended with sewage sludge at different rates and reported that metal concentrations in the plants displayed a plateau response. However, concentrations of total or free metals in the soil solution did not display a similar plateau response; hence they contended that the pre-requisite for determining that metal uptake by plants was limited by sludge chemistry was not met. It was concluded that evidence of a sludge driven plateau response in metal uptake by plants will only be obtained when studies have found a good hyperbolic relationship between soil solution metal concentrations with increasing sludge application rate and can link this to a plateau response in plant uptake of metals. Plant physiological factors such as a molecular block to uptake or translocation of metal is involved in regulating metal uptake or saturation of the metal uptake mechanism at the root surface were responsible for the plateau in plant metal concentrations observed in their study.

Zaier *et al*. (2010) evaluated the capacity of canola to remove metals from soils amended with sludge. Seedlings were cultivated in presence of sludge combined with and with out EDTA in the soil. Sludge increased significantly biomass production and an increase in Pb, Zn and Mn shoot concentrations of canola. EDTA application does not affect significantly plant growth; however, as a chelator it enhanced shoot metals accumulation. It was concluded that canola can be used for the decontamination of affected soils and that the EDTA addition increases the ability of canola to accumulate heavy metals. Growing of canola on sewage sludge-amended soils may possibly be a solution to reduce toxic metal concentrations in soils and avoid the risk of groundwater and food heavy metal-induced contamination.

Marchiol *et al.* (2004) investigated the phytoextraction potential of canola and radish, the crops were grown on a multi-metal contaminated soil in a pot-experiment and the results showed that both plants were moderately tolerant to heavy metals and radish was more tolerant than canola. It was concluded that both crops could possibly be used with success in marginally polluted soils where their growth would not be impaired and the extraction of heavy metals could be maintained at satisfying levels. Rasmus *et al*. (2009) reported no significant increases in concentrations of Cu, Fe, Zn and Mn in oats shoot, but a considerable increase in levels of Ni and Co in oats shoot for oats crop grown on acid sulphate soil with varying geochemical characteristics. Bjerre and Schierup (1985) studied the influence of adding heavy metals into soil on the level of uptake of these metals by oat. The results indicated that addition of Cd increased the concentrations of Cd in all oat plant parts but lower concentrations of Cu, Zn and Pb in the plant shoot were observed. Uptake of metals by oats was dependent on soil type, with the greatest uptake recorded from sandy soil, generally the total metals uptake was lowest from organic soils as compared with when Cd was added separately.

Overall, bioavailability of heavy metals from biosolids to plants can be affected by several factors; these may include, individual trace elements, physicochemical properties of the soil and sludge/biosolids characteristics. The transfer of heavy metals to plants also differs with plant species, varieties and the different plant parts. In most cases, heavy metals tend to concentrate in plant leaves rather than in the grain or fruit parts. Long term studies conducted by a number of researchers showed that the sludge time bomb hypothesis which states that the decomposition of biosolids organic matter through time may release soluble forms of heavy metals into the soil and their subsequent transfer into the plant system did not occur. Several researchers also indicated that plant uptake of heavy metals from land applied biosolids was not linear, but showed a plateau response where heavy metals in plant reached maximum and levelled or decreased following biosolids applications.

2.7. Nutrients in biosolids

Nutrients in biosolids are usually not readily available to the plants and are released slowly, which may enhance nutrient uptake efficiency and, as a result, reduce risks associated with nutrient losses. Nitrogen, phosphorus, and sulphur are the major plant nutrients contained in biosolids and are utilized to the greatest extent by plants (Epstein, 2002) and this review focuses mainly on the major nutrients (N, P and S) in biosolids.

2.7.1. Nitrogen in biosolids amended soil

Nitrogen is a growth limiting factor for many of the plant species grown on biosolids amended soils; it is found in biosolids in both organic and inorganic fractions, the quantity being dependent on the method of sewage and sludge treatment processes (Smith, 1992; Smith, 1996).

The long term value of biosolids applications could be the provision of supplying slow conversion (mineralization) of organic-N to inorganic-N (NO_3-N and NH_4-N) fractions during the growing season of the crop (Erhart ***et al.***, 2005).

Table 2.7 describes the effect of loading different rates of organic wastes on the levels of total and extractable nitrogen in soil. A number of researchers (Hernandez ***et al***. 1991; Harrison *et al.* 1994; Banuelos *et al.* 2007; Tarrason *et al.* 2007) reported increased concentrations of total nitrogen following application of digested fresh biosolids, composted sludge, municipal sewage sludge and anaerobically digested sewage sludge under field conditions.

Significant increases in NO_3-N levels because of biosolids applications were also reported by Hernandez *et al.* (1991) ; Stewart *et al.* (1998) ; Rodrigue *et al*. (2003); Wang *et al*. (2003) ; Bakhsh *et al*. (2005) ; Correa *et al*. (2006) in field and pot experiments. However, Tarrason *et al*. (2007) found a decrease in NH_4-N and NO_3-N fractions following the applications of anaerobically digested sludge on a calcareous loamy soil under field conditions. Parkinson *et al*. (1996) reported no change in NO_3-N after the applications of municipal solid waste and green waste compost on a fine loam soil under field conditions.

Table 2.7 Effect of various organic amendments on total and extractable nitrogen in amended soil under different field and glass house conditions.

Organic waste types	Experimental details	Soil	Crop types	Effect on total and extractable nitrogen	References
Anaerobically-digested fresh sludge composted sludge and thermally dried sludge	10 t ds/ha surface applied in a field	A	Rye grass , orchard grass and tall fescue	Decrease in NH_4-N and NO_3-N, eventual increases in TN	Tarrason *et al.* (2007)
Aerobic or anaerobic sewage sludge, Chicken manure,	Added to raise soil oxidisable carbon by 1.5% under field	C	Maize and barley	TN increased for all organic waste types	Hernandez *et al.* (1991)
Anaerobic municipal sewage sludge	0, 2.4, 17, and 60 t ds/ ha/year surface applied under field	P	Monterey pine	No NO_3-N and NH_4-N leaching 2.4 t/ ha/ yr application for two yrs. Repeated applications.	Arbestain *et al.* 2005)
Aerobic or anaerobic sewage sludge. Chicken manure,	Added to raise soil oxidisable Carbon by 1.5%	C	Maize and barley	NO_3-N increased for all organic waste treated plots	Hernandez *et al.* (1991)
Municipal sewage sludge	500 t ds/ha incorporated under field condition	B	Lombardy popular, Douglas fir and ponderosa pine	Three fold increases in TN	Harrison *et al.* (1994)
Class A biosolids	1.9, 5.8 and 11.7 t ds/ha incorporated under field	I	apricot	Significant increases in TN	Banuelos *et al.* (2007)
Municipal solid waste compost	90 t ds/ha incorporated annually for three years in a field	E	Corn	Higher NO_3-N leaching in 1st and 2nd year but not in the 3rd year	Hartl *et al.* (2003)
Biosolids, coal ash and compost	205 g/pot incorporated in a pot	D	Sunn hemp and Sudan grass	25 fold increase in NO_3-N in leachate, low NO_3-N in yard compost	Wang *et al.* (2003)

Table 2.7 continued Effect of various organic amendments on total and extractable nitrogen in amended soil under different field and glass house conditions.

Anaerobically digested biosolids	120 kg/ha incorporated under field condition	J	soybean	Low NO_3-N leaching due to high denitrification	Angle *et al.* (2003)
undigested liquid, digested liquid and dewatered, anaerobically digested cake	4, 8, and 16 t dry solid /ha incorporated in a tube study	I	rye grass	Increased NO_3-N in plants due to each sludge type applications	Smith and Tibbett (2004)
fresh sewage sludge, composted sludge, limed sludge, heat-dried sludge and solar-irradiated sludge	0.5–8.0 t/ dry solid/ha incubated under glass house	K,L	No crop	NO_3-N levels increased due to biosolids, but NO_3-N leaching was very low	Correa *et al.* (2006)
Fresh sludge	8, 16 and 24 t/ dry solid/ha incorporated in field	M	maize	significant increase in NO_3-N following biosolids applications	Rodrigue *et al.* (2003)
Compost	0, 22 and 44 t/ ha incorporated under field condition	N	broccoli	Positive correlation between broccoli weight and leaf N to soil nitrate-N, but low NO_3-N in compost treated plots	Stamatiadis *et al.* (1999)
compost	7.5kg/10m2 surface applied in a field	D	lettuce	significant correlation between leaf and soil nitrates	Patriquin *et al.* (1993)
Municipal solid waste, sludge & green waste compost	15,30 and 50 t/ha fm	G	-	NO_3-N in compost treatments were not different from control	Parkinson *et al.* (1996)
Municipal solid waste compost	50 and 100 t/ha fm and 75 and 150 kg N/ha incorporated under field	F	Corn	NO_3-N in compost treated plots were the same as fertilizer plots	Rodrigue *et al.* (1996)
liquid swine manure and urea ammonium nitrate	82-262 kg N/ha incorporated in under field condition	H	Corn and soybean	increased NO_3-N in tile due swine manure applications	Bakhsh *et al.* (2005)
Spent mushroom substrate	0, 20, 40, or 80 t/ha (moist) incorporated under field condition	-	potato, cabbage & sorghum bicolor	increase in soil inorganic N	Stewart *et al.* (1998)

The superscripts calcareous loamy(A), coarse textured (B), calciorthid(C) gravelly loamy and calcareous,(D), loamy sand (E), loamy clay (F), silty loam (G), fine loam (H), sandy loam (I), coarse loam (J) , podosol (K), clay ferrosol (L), lime loam Ah horizon (M) and silty clay (N) attached on soil, refers to various soil types respectively.

2.7.2. Phosphorus in biosolids amended soil

Beneficial reuse of biosolids and manures based on crop nitrogen requirements, supplies phosphorus in excess of crop needs. Excess soil phosphorus is not harmful to plants when biosolids are used for nitrogen fertility (Peterson *et al*., 1994).

However, off-site migration to aquatic systems is a major concern because phosphorus is the limiting nutrient in most freshwater systems (Sharpley and Beegle, 1999). Phosphorus moves from agricultural fields either in a dissolved form or attached to soil particles. When soil phosphorus levels are not excessive, up to 90% of the phosphorus transported from cropland is bound to soil particles (Sharpley and Beegle, 1999). Thus, erosion control measures prevent significant off-site phosphorus movement. However, some agricultural fields have soil test P levels in the high and very high category, and off-site transport of soluble phosphorus can be important.

The impacts of loading a range of biosolids types on the concentrations of total and extractable soil phosphorus in field experiments are summarized in Table 2.8 and 2.9. Several workers (Stewart *et al*. 1998; Penn and Sims 2002; Arbestain *et al*. 2005; Alves *et al*. 2006; Courtney and Mullen 2007; Kidd *et al*. 2007) recorded significant increments in Olsen-P levels following different biosolids application on various soil types. Increases in total soil phosphorus due to the applications of municipal sewage sludge were also reported by Harrison *et al*. (1994).

A considerable amount of Al and Fe present in many of the biosolids because of chemical additions to the waste water and sludge treatments processes or because of water treatment residuals are discharged to sewers. For biosolids with too little Al and Fe, chemical addition (Moore *et al*., 1999) or co-application with water treatment residuals (O'Conner and Elliot, 2000) can dramatically increase phosphorus fixation and in turn eliminate concern over phosphorus leaching in biosolids amended soil. Soon and Bates (1982) also observed an increase in phosphorus solubility due to a decrease in soil pH following addition of Fe treated sludge.

Table 2.8 Effect of anaerobically digested various organic waste amendments on total and extractable phosphorus in soil under different field and glass house conditions

Organic waste types	Experiment details	Soil	Crop	Effect on total and extractable phosphorus	References
Anaerobically digested sewage sludge	154-271 kg P/ha for cotton wood and 195-350 kg P/ha for switch grass six years surface applications (field).	O	switch grass & cotton wood	HCl soluble fraction of -P transformed to more labile forms ($NaHCO_3$–Inorganic P) because of the relatively low pH of the biosolids-amended soil.	Thomson *et al*. (1999)
Anaerobic municipal sewage sludge	0, 2.4, 17, and 60 t ds/ ha/year which was equivalent to 0, 1.2, 8.4 and 29.8 kg Olsen P/ha surface application (field)	P	Monterey pine	Highest Olsen-P (1116 µg/g) observed at the 60 t/ha sewage sludge amended plots. Total P in foliar tissue of the tree was higher than control plots.	Arbestain *et al*. (2005)
Anaerobically digested, aerobically digested undigested biosolids and a biosolids produced by a biological removal technique and poultry litter	200 kg P/ha biosolids and poultry litter and 45 kg P/ha fertilizer incorporated (field)	G,I	Corn, soybean & alfalfa	Biosolids produced with a biological nutrient removal technique process caused the highest increases in extractable soil P and runoff dissolved reactive phosphorus. Biosolids produced with iron only had the lowest extractable P and caused the lowest increase in extractable soil P and runoff dissolved reactive P. Addition of Fe to soils through biosolids may be beneficial in preventing P losses through runoff by increasing soil P sorption capacity.	Penn and Sims (2002)
Anaerobically digested sewage sludge	Incorporated in a field experiment	E	Corn	Soil Fe and Al oxide content was increased. This increased P retention, occurred through sorption and occlusion by the oxides.	Soon and Bates (1982)
Anaerobically digested sewage sludge	1000 t ds/ha biosolids which was equivalent to 3405 kg P/ha was added in a lysimeter experiment	Q	-	A very high level of Olsen-P in the sludge amended soils throughout the experiment and a very low rate of PO_4^{3-} leaching, indicating P was adsorbed rather than solubilised in this soil/sludge mix.	Zhang *et al*. (2004)

The superscripts refer to various soil types and textures, for coarse textured (B, loamy sand (E), silty loam (G), fine loam (H), sandy loam (I), , Mollisol (O), Cambisol (P), coarse sandy(Q), medium textured and dystrophic red lotosol (R), attached on soil, refers to various soil types respectively.

Table 2.8 continued Effect of aerobically digested and other various organic waste amendments on total and extractable phosphorus in soil under different field and glass house conditions

Organic wastes	Experiment details	Soil	Crop	Effect on total and extractable phosphorus	References
Aerobically digested biosolids	5, 10, 20, and 40 t ds/ ha incorporated in a field, biosolids had 18 $mmole_c\ dm^{-3}$ resin extractable P concentration	R	sorghum bicolor	Increased soil P at 40 t/ ha rate, except for the treatment with 20 t/ ha.	Alves *et al.* (2006)
Spent mushroom compost ,forced aeration compost and fertilizer	0, 25, 50 and 100 t ds/ha equivalent to 57, 114 and 289 kg P/ha were incorporated in a field experiment	-	Barley	Composts significantly raised plant-available phosphorous levels.	Courtney and Mullen (2007)
Spent mushroom substrate	0, 20, 40, or 80 t ds/ha (moist) , equivalent to 56, 112 and 224 kg P/ha were incorporated into soil (field)	-	Potato, cabbage & sweet corn	Increase in Olsen extractable P from 13.8 mg/L - 63.5 mg/L at 80 t/ha spent mushroom compost rate.	Stewart *et al.* (1998)
Digested sewage sludge incorporated in pots	Control soils limed with 2 t $CaCO_3$ /ha and fertilized 150 kg P/ha, 200 kg K/ha as KH_2PO4 and 100 kg N/ha as NH_4 and NO_3 at a biosolids disposal site.	I	Maize, Alpine alyssum & gum cistus	10 fold increase in Olsen-P, pH and CEC.	Kidd *et al.* (2007)
Municipal sewage sludge	500 t ds/ha which is equivalent to 9100 kgP/ha was incorporated a field experiment	B	Lombardy popular, Douglas fir & ponderosa pine	Five fold increase in total phosphorus levels.	Harrison *et al.* (1994)
Compost and manure	8.58 t ds/ha (compost) and 14 t ds/ha (manure) equivalent to 53.6 kg P/ha and 10 kg P/ha , respectivelyfor 5–6 years incorporated in a field	H	potato	Increases in inorganic and organic soil P. Decreasing P solubility as pH increases from 5 to 7 which were attributed to increasing hydrolysis of exchangeable Al and subsequent reaction of the hydrolytic species with soluble P.	Erich *et al.* (2002)

The superscripts refer to various soil types and textures, for coarse textured (B), loamy sand (E), silty loam (G), fine loam (H), sandy loam (I), , Mollisol (O), Cambisol (P), coarse sandy(Q), medium textured and dystrophic red lotosol (R), attached on soil, refers to various soil types respectively.

Most organic P compounds have a reduced solubility in acidic soils, and become stabilized by association with clays and humic compounds (Goring and Bartholomew, 1952; Anderson and Arlidge, 1962). This is evident by the negative correlations between bicarbonate-extractable organic P and soil pH (Tiessen *et al*., 1984**),** and it affects the relative bioavailability of bicarbonate-extractable organic P from a range of soil types.
The effect of increasing pH on soluble P is dependent on soil mineralogy as well as initial and final soil pH. Soils in which aluminosilicates are the predominant P adsorbents may show a decrease or no change in P solubility with increasing pH **(**Traina *et al*., 1986).
Erich *et al*. (2002**)** observed a decreasing P solubility as pH increases from 5 to 7 which was attributed to increasing hydrolysis of exchangeable Al and subsequent reaction of the hydrolytic species with soluble P. Any soil which contains less than 2 mmol Al/kg is unlikely to show a decrease in soluble P with an increase in pH up to 7 (Traina *et al*., 1986).
Otabbong *et al*. (1996) investigated the effects of pH on the availability of P from municipal sewage sludge and found that solubility of P in sludge relative to single superphosphate fertilizer decreased in response to increasing soil pH **(**as a result of liming).
Al phosphates and Fe phosphates are the predominant P minerals in soils with pH levels below about 6.5 (Havlin *et al*., 1999). The solubility of these minerals decreases at lower pH, directly opposite to the solubility for calcium phosphates. Therefore, P is most available around pH 6.5, because at lower pH levels, P retention is high due to Al-P and Fe-P precipitation, and at higher pH levels, Ca-P minerals precipitate.
In summary, the use of different biosolids at various rates with a range of crops and soil types increased the N and P status of the soil. Excess N and P applied to soils can be a source of pollution in both ground-and surface water. Nitrate can move through the soil profile into groundwater resources, and P and N in runoff and eroded particles can enter surface waters which may result in eutrophication. The likelihood of P run off is very dependent on type of soil, biosolids treatment process and pH of soil and biosolids.

The preceding discussion has centred on biosolds that have not undergone further processing after aging. When biosolds are co-composted with vegetative materials the organic composition of the resultant product is likely affect P availability.
Xiong *et al*. (2010) investigated the effect of adding wood sawdust and maize straw on sewage sludge during co-composting on the formation and molecular transformation of humic substances. The findings indicated that the composting process increased humic

acids and lowered the levels of fluvic acids, in particular the wood sawdust and maize straw increased the humic acid by 25 % and 16 % respectively. It was concluded that organic bulking agents such as wood sawdust improved Cd and Cu complexing ability of humic acids.

Smidt *et al*. (2008) investigated the influence of adding lignin on the humification process during co-composting of different lignin sources under a laboratory experiment

The preliminary results showed that addition of 2-5% of lignin powder improved humic acid formation; however, biodegraded wood added during the co-composting process did not produce higher humic acid contents in the short time laboratory scale composting.

It was concluded that due to economic reasons addition of black liquor from pulp production with no pre-treatment could enhance compost quality.

Wang *et al*. (1995) examined the impact of adding humic acids on phosphorus fertilizer transformations in an alkaline soil under laboratory condition.

Results of the study demonstrated that addition of humic acids to soil with P fertilizer considerably increased the amount of water soluble phosphate strongly retarded the formation of occluded phosphate and increased P uptake and yield by 25%.

It was concluded that addition of humic acids reduces phosphorus fixation and increased water soluble phosphorus where wheat yield and phosphorus uptake increased due to the addition of humic acids.

Singh *et al*. (1990) examined the production of humic substances and their ability to retain phosphorus and calcium liberated during composting of wheat straw with two types of low-grade rock phosphate materials. The results indicated that quantities of humic acids increased as composting time increased, but fulvic acids production decreased after 30 days of composting. The addition of Mussoorie phosphate and Hyperphos hindered humic acids production and improved fulvic acids production. The production of humic acids and their retention capacity for phosphorus and calcium were greater in the presence of Mussoorie phosphate than Hyperphos material.

Humic substances, particularly fulvic acids, adsorb a considerable amount of calcium and release H^+ ions which would solubilize rock phosphate. Humic substances released during composting may possibly ensure the reprecipitation of solubilised phosphorus and calcium by complexing both ions and creating a sink in the system for further dissolution of rock phosphate.

Sathiyabama *et al*. (2003) investigated the influence of adding potassium humate on nutrient release, organic carbon and cation exchange capacity of an Alfisol in a laboratory incubation experiment. The application potassium humate increased the release of nitrogen, phosphorus and potassium and showed a linearly trend. Nitrogen and phosphorus increased for two months period, but potassium release reached plateau on forty five days after incubation

Significant increases in organic carbon and cation exchange capacity of the soil was also noted at the end of the composting period.

The literature suggests that composting biosolids blended with various organic wastes significantly increased the quantity of humic acid produced and reduced the levels of fulvic acids. Humic substances released during composting may solubilise phosphorus and increase its availability to plants.

Phosphorus fertilizer equivalency of biosolids

Unlike N contained in biosolids, the bulk of biosolids P exist in inorganic forms and hence, the inorganic P form determines the plant available P in biosolids treated soil soils (Fine and Mingelgrin, 1996). Similar to conventional fertilizer P, the forms of P which are chemically bound to Fe or Al in biosolids (O'Connor ***et al***., 2004) are the major P forms in biosolids amended soil (Chang et al., 1983, Maguire et al., 2000, Su et al., 2007, Folle ***et al***., 1995). Due to the greater reactivity of Al, the level of Al-P is higher than Fe-P in biosolids amended soils (Folle ***et al***., 1995).

In order to determine appropriate loading rates to satisfy crop requirements and prevent losses to the environment, Prichard (2005) examined the relative effectiveness of P in anaerobically digested dewatered biosolids compared to inorganic P fertiliser in field and laboratory experiments on a sandy soil with low P sorbing capacity under Western Australian condition.

By comparing shoot uptake of P at 33 days after sowing of wheat crop, the P equivalency of biosolids relative to Monocalcium phosphate was 118% in the first crop, 33% in the second crop and 16% as effective in the third crop. A field investigation was also conducted using wheat and lupins in a lateritic podzolic soil, compared to freshly applied triple superphosphate, the first year P equivalency determined by grain harvest was 67 – 79% relative to triple superphosphate with a residual value of 47% in the second year.

During the field experiment, surface application of biosolids with no incorporation decreased the plant available P compared to incorporation, by approximately 40% indicating the importance of placement and physical factors impacting the uptake of P by plants (Pritchard, 2005).

Barry *et al*. (2006) conducted four field trials in Queensland to investigate the fate of P in anaerobic and aerobic biosolids amended soil. The total P loading rate was at 1 NLBAR (all > 400 kg P ha^{-1}) exceeding of typical fertiliser applications rate of 20 – 40 kg P ha^{-1}. The results showed that biosolids application increased crop P uptake and crop responses were site dependent, consistent with background soil P concentrations. The cumulative P removed by the crop from biosolids at each site was between only 1.5 – 5 % of the total P added, which may indicate concerns about the potential for leaching of residual P in the soil profile

Under Western Australia acidic sandy soil with low extractable P condition, Rigby *et al*. (2010) showed lower bioavailability of alum treated sludge. The results indicated that the alum biosdolids applied at rates between 0.5 - 4.5 NLBAR provided insufficient P for wheat growth; shoot tissue from alum sludge treatments was deficient in P, whereas wheat fertilised with inorganic fertiliser P had adequate shoot P concentrations. In the second year of the experiment, the availability of P in alum sludge amended soil did not improve, with barley growth and nutrient uptake showing a similar pattern to first year observations.

The availability of biosolids P depends on soil type, pH, Fe and Al content and biosolids treatment technique. The availability of biosolids P is in the range – 20 – 130% as available as inorganic fertiliser P. The majority of P forms is bound to Fe and Al in the biosolids and is not readily extractable and thus P in biosolids is generally less available than inorganic fertiliser P and P from organic manures. During wastewater treatment, chemical treatment techniques employed to remove P from waste water, further reduce P availability in biosolids. Compared to a mean of 49% (range 34 – 70%) with chemical treatments employed to remove P, the mean P equivalency of anaerobically digested biosolids with no chemical P removal is 56% (range – 22 – 100%). Anaerobically digested biosolids generated from biological phosphorus removal processes had a greater mean P equivalency of 76%.

Heat drying decreases P availability due to a reduction in soluble P forms. Lime treated biosolids may also influence P availability, depending on their influence on soil pH.

Biosolids type and soil type interactions is crucially important in determining P availability as P binds to Fe and Al compounds at low soil pH values and Ca at high pH values, but solubility usually increases with increasing pH. The phosphorus saturation index (PSI) is a measure of the ratio of oxalate extractable P to the sum of oxalate extractable Fe and Al, when biosolids are added to soil the PSI of the soilbiosolids blend will determine the P availability. A PSI greater than one indicates that there is labile P present that is not associated with Fe and Al precipitates and a phosphorus saturation index less than one indicates that there is little labile P (O'Connor et al., 2004).

The availability of biosolids P may possibly be dependent on physical factors such as placement of biosolids. Phosphorus has low solubility and is generally not mobile in soil.

Plant uptake of P from biosolids

Differences in the phytoavailability of P in various biosolids are crucially important for the efficient utilization of biosolids P for agronomic uses (O'Connor *et al*., 2004).

Barbarick *et al*. (2004) applied composted biosolids at rates of 0, 5, 10, 20, 40, and 80 t/ha to a harshly burned, formerly forested site to enhance soil C and N levels and observed that soil and plant tissue P concentrations of stream bank wheatgrass increased with increasing application rates of biosolids which significantly enhanced forage quality and improved nutrient cycling.

Kidd *et al*. (2007) conducted a laboratory experiment using sandy loam soils collected from a field where dry digested sewage sludge from a wastewater treatment plant had been applied every two years for more than 10 years. The control soils were limed with agricultural limestone (applied at a rate equivalent to 2 t $CaCO_3$ /ha) and then fertilized with 150 kg P/ha, 200 kg K/ha as KH_2PO_4 and 100 kg N/ha as NH_4NO_3. The dry digested sewage sludge amended soils were neither limed nor fertilized. Soils were adjusted to 80% of their water holding capacity and left for two weeks to attain equilibrium. Two wild plants (*Alyssum serpyllifolium* and *Cistus ladanifer*) and a maize crop (*Zea mays*) were planted. The results indicated that P uptake was significantly greater in plants growing on dry digested sewage sludge amended soils and this was most pronounced in the hyperaccumulator, *Alyssum serpyllifolium*. However, tissue P content in maize did not differ greatly between plants grown on control or amended soils, suggesting that P was added in excess and under maize cultivations there is a higher risk of P loss through leaching.

Marschner *et al*. (2007) conducted a commercial fertilizer experiment to assess the role of soil type on growth, P uptake and rhizosphere properties of wheat and canola genotypes in an alkaline soil with low P availability under field conditions. Phosphorus (200 mg P /kg) was added as $Ca_3(PO4)_2$.

Shoot and root dry weight, root length and shoot P content were greater in the two canola genotypes than in wheat. Canola genotypes had a greater dry weight and took up more P than the wheat genotypes. Furthermore, growth and P content was improved by P addition in the canola but not in the wheat genotypes. The canola genotypes had a greater root length compared to the wheat genotypes, leading to a greater soil volume exploited. The importance of root length was also evident in the strong correlation with P content, particularly in canola. Thus, despite similar concentrations of available P in the rhizosphere, total shoot P content was greater in the canola than wheat genotypes and concluded that the greater soil volume utilized by the canola crops may explain their capacity to respond to P addition. Canola crops have also been shown to increase P solubility by release of organic acid anions into the rhizosphere and/or changing the rhizosphere pH (Hoffland *et al*., 1989; Hoffland, 1992; Gerke and Meyer, 1995; Imas *et al*., 1997).

Phosphatase enzymes discharged by the roots of most plants have the ability to decompose organic P enhancing P availability by plants (Tarafdar and Claassen, 1988), P mobilization through chelation and ion exchange could also be accountable for high phosphorus uptake by plants.

Rendel *et al*. (2007) investigated the effect of management systems and crop rotations on soil P fractions and selected soil properties in a volcanically derived Ultisol under field conditions. The study evaluated two tillage systems, no tillage and conventional tillage, and two crop rotations, oat-wheat and white lupin-wheat in order to determine the effects of such management in the lability of P in the soil. The study showed that significantly higher labile and relative soil labile P forms in the oats-wheat rotation than in the lupine-wheat rotation.

Rotations with oats have been shown to have more relatively labile P; hence lupin cropping depleted more non labile P to relatively labile P, suggesting that lupine is able to take up P from this stable pool which could be related to lupine's ability to enhanced root release of organic acid anions at limiting P supply (Horst *et al*., 2001). It was also noted that soil pH was positively correlated with labile P fractions, but negatively correlated with the relatively labile NaOH-P_o. The results suggest that the enhanced phosphatase activity of lupine caused only a slight accumulation of labile P_o in subsequent wheat and a depletion of non-labile P, but oats rotation accumulates more relatively labile organic P, protecting it from becoming unavailable for plants. This suggests that the inclusion of plants like oats or lupine in crop rotations as well as tillage cropping, could prevent P accumulation in soils under fractions with lesser availability to plants.

In general, several researchers reported increases in P uptake by different plants for some of the used for land applications, and P uptake depends on the forms and characteristics of P in the biosolids. The uptake of P differs with plant species, since the roots of various plants release different levels of phosphatases into the soil which can mineralize the organic P further increasing P availability and its subsequent uptake.

2.8. The response of various crops to fertilizer and biosolids amendment

The yield response of a range of crops to different types of biosolids and sludge applications is summarized in Table 2.9. Several researchers reported that the addition of biosolids, sewage sludge and municipal solid waste compost to crop land increased the growth and yield of wheat, barley, maize and soybean (Hernandez *et al*., 1991; Zhang *et al*., 2000; Qasim *et al*., 2001; Karin *et al*., 2003; Antolin *et al*., 2005 and Kim *et al*., 2007).

Dowdy *et al*. (1978) reported that the increase in crop yields due to biosolids applications frequently exceeded that of conventionally fertilized control plots.

Such increases in the yield of crops were due to the ability of biosolids to increase the organic matter content, water holding capacity of soil and provision of available plant nutrients to the crops; however the responses of various crops to biosolids applications would also depend on external factors such as rainfall, soil and crop types and biosolids characteristics, these factors also determine the rates of biosolids applications. Therefore, further study that focuses on the land application of biosolids under crop rotation would be necessary, particularly taking into account site specific soil, plant and biosolids characteristics.

Yasari *et al.* (2008) evaluated the influence of conventional fertilizer (NPK) applied together with S and Zn, singly or in combination on yield and nutrient uptake of canola. The results showed that higher seed yield were obtained at the higher fertilizer application rate which coincided with higher concentrations of nutrients (N, P, K, S and Zn) in canola leaf at flowering stage indicating significant levels of translocation of nutrients at various stages of plant growth and with higher number of pods per plant.

Table 2.9 Effect of various organic waste amendments at different rates on the yields of a range of crops under glass house and field conditions

Crop types	Organic amendment rates	Effect on crop yield	References
Herbaceous mixture of plants	Anaerobically digested biosolids (40, 80 and 120 dry t/ha)	Significant increments were found in total plant cover and total biomass production.	Walter *et al*. (2000)
Maize and barley	Aerobic or anaerobic sewage sludge, Chicken manure. Added to raise soil oxidisable carbon by 1.5%	Yield of maize and barley increased	Hernandez *et al*. (1991)
Soybean	Dewatered-digested sludge and composted sludge, Alkaline stabilized sludge	Increase in soybean seeds yields in limed sludge-treated soils, with the highest yield in the composted treatment, followed by the dewatered-digested sludge and alkaline stabilized sludge	Kim *et al*. (2007)
Wheat and barley	Air dried biosolids at 2-10 dry t/ha application	Improved dry biomass and yield of wheat and barley	Karin *et al*. (2003)
Cotton	0, 10, 30 and 50 dry t/ha sewage sludge in a clay loam soil	Increased cotton yield from 2.47 to 3.35 t/ha	Tsadilas *et al*. (2005)
Tall fescue	Sewage sludge (5.6 dry t/ha) given three times over a 2-yr	30 % higher yield increased	Boswell (1975)
Barley	Sewage sludge (15 dry t/ha)	Grain yield of barley increased significantly under repeated sewage sludge application	Antolin *et al*. (2005)
Apple tree	Sewage sludge (0, 12.5, 25, 50 and 75 dry t/ha) and barnyard manure (47 dry t/ha)	Sewage sludge amendment increased the fruit yield	Bozkurt and Yarilgac (2003)
Flax (Linum usitatissimum).	Sewage sludge amendment (2:1 and 10:1 v/v soil and sewage sludge ratio)	Greater biomass in the 2:1 soil: sludge ratio, and a significantly greater number of stems/plants with increased seed production/plant and seeds of higher average seed weight.	Tsakou *et al*. (2002)
Maize	Sewage sludge (0, 10, 20, 30, 40, and 50 dry t/ha), NPK (120:90:30 kg/ha) and farm yard manure (3.5 dry t/ha) applications	Maximum shoot and root dry weights were reported at 20 t/ha sludge application	Qasim *et al*. (2001)
Carrots and chard	Municipal biosolids	Significant increase in yield more than those grown on recommended NPK.	Nielson *et al*. (1998)
Barley and wheat	Municipal solid waste compost was applied at rates of 50, 100 and 200 t/ ha (fresh matter)	Barley yields were 270 % of the untreated control in the 50 t/ha treatment, wheat yields 170 % and canola yields 148 %	Zhang *et al*. (2000)

2.8.1. The use of canola in crop rotation

Canola not only provides good returns but also it improves the yield of other crops. The benefits of canola as a break crop particularly in arid regions of New South Wales was reported by Angus *et al*. (1989), where the efficiency of applied N was consistent and usually greater for wheat following canola than wheat following other oilseeds.

The rotational advantage of using canola with cereals was the main factor that contributed for the rapid adoption of canola by famers (Norton *et al*., 1999). Because of the rotational advantage of canola, growers rapidly adopted and incorporated the crop into their production system. Several farmers also realized the need for additional N for canola as management practice (Hocking *et al*., 1999) and extended the management practice to wheat crops.

The magnitude of the break crop effect due to canola is significant to the grains industry. Experimental data derived from a Victorian survey of 226 wheat crops shows that Wheat grain yields of 3.9 t/ha were recorded where canola was the previous crop, compared with wheat on fallow which was 3.2 t/ha wheat grain yield (Norton et al., 1999). Angus *et al*. (2001) summarized the results of several crop sequence experiments across the grain producing areas and reported an increase of about 20 % in wheat grown after canola compared to wheat after wheat. Similar results were observed in 14 on-farm experiments in Southern New South Wales, where wheat after canola produced a 21 % higher yield than wheat after wheat (Angus *et al*., 1999).

The nature of the break crop benefit due to wheat after canola was the subject of research in Australia during the 1990s (Kirkegaard *et al*., 1994; Angus *et al*., 1998; Kirkegaard *et al*., 1998; Kirkegaard *et al*., 2000). Although several other factors could be aiding subsequent wheat growth, such as differences in organic matter cycling (Angus *et al*., 2001), it is clear that canola does provide a significant benefit to subsequent wheat crops.

Angus *et al*. (1999) estimated a benefit of $147 per hectare for a two year canola/wheat rotation over a wheat/wheat rotation. Based on the yield, costs and prices presented, 27 % came from the canola, while the balance came from the subsequent wheat crop. Angus *et al*. (1999) noted that wheat could substitute into rotations when its price was about 60% of the price of canola. Current on-farm prices (with various discounts) for canola ($420/t) and wheat ($250/t) (ABARE, 2003), place the difference between the two rotations at about $30/ha over 2 years, mainly due to the relatively high wheat price compared to the canola price. A similar price ratio (60 %) was estimated by Scott et al (1999), using a whole farm linear programming framework, who concluded that when wheat was $150/t, canola could constitute 25 % of the cropped area on a southern New South Wales farm when canola prices rose above $340/t with a yield of around 1.8 t/ha.

Guo *et al*. (2006) conducted a field experiment to investigate the effect of crop rotation and tillage on blackleg disease in canola rotated with wheat and flax crops. Rotation and tillage significantly reduced blackleg disease in terms of the number of infected plants, infected leaves and lesions per plant, and percentage of leaf coverage with lesions. The number of infected plants, infected leaves and lesions per plant, and percentage of leaf coverage with lesions were lower in canola when rotated with wheat.

Crop rotation takes advantage of the fact that plant pathogens crucial on one crop may not cause problems on another crop (Kharbanda 1999). Suitable crop rotation lengthens the time between similar host plants; as a result of this, populations of pathogens will decline in crop rotation to no host crops with tillage, and hence this situation could significantly reduce the incidence of blackleg disease of canola.

2.8.2. The response of oat to fertilizer

Weightman *et al*. (2004) examined the effects of N and water availability on grain quality of two UK winter oat varieties over two seasons at two sites. Nitrogen fertilizer treatments comprised three rates and two forms of fertilizer (ammonium nitrate and foliar urea). Ammonium nitrate was applied as a split application with 40 kg N/ha and foliar Urea was applied as an aqueous spray. The findings indicate that ammonium N fertilizer had significant effects on grain yield and quality. Yields increased at the first applied ammonium nitrogen level (40 kg N/ha) representing standard (optimal) agricultural practice at those sites, then decreased at the highest (100 kg N/ha) ammonium nitrogen fertilizer level. In five out of the six data sets, the maximum yield was recorded with the combination of the optimal ammonium nitrogen fertilizer rate, plus application of 60 kg N/ha as foliar urea.

Entz *et al*. (2004) examined the cultivar responses of a short stature oat and a tall oat under different crop rotation (grain legume or oilseed as a previous crop) and various N fertilizer rates (0, 40, and 80 kg N/ha) in 1999 and four rates (0, 40, 80, and 120 kg N/ha) in 2000. It was observed an increase in grain yield as nitrogen application rates increased. Plant heights and lodging scores were higher in the pea rotation and increased with increasing nitrogen rates. Increased N fertilizer rates increased N accumulation at all their sampling sites. Previous crops only affected N uptake in 2000, where oats following pea had greater N accumulation at all sampling times than oat following flax.

Hamill (2002) concluded that a total N supply of 115 kg N/ha was optimum for maximum yield of oats in Manitoba (Canada) and optimum N supply may differ between oat cultivars. While oats yield response to nitrogen fertilizer is well documented, there is limited information on the influence of organic N sources such as rotational legume crops. The inclusion of a green manure legume in crop rotations can increase residual soil N levels and increase seasonal nitrogen availability to following crops (Badaruddin and Meyer 1990; Stevenson and van Kessel, 1996).

Hence, N management is important in the production of oats for yield optimization and lodging control (Brinkman and Rho, 1984; Marshall *et al*., 1987).

The application of N to oats directly affects the various yield components, such as panicle density and kernel number, as well as dry matter production and harvest index. The relative contribution of each yield component in response to increased nitrogen level will vary depending on the levels of N used and environmental conditions (Brinkman and Rho, 1984; Marshall *et al*., 1987; Anderson and McLean, 1989; Hamill, 2002).

In general, yield responses of various crops were affected by the type of biosolids and sludge treatment processes with the exception of the oats, the increases in the productivity of the various crops following biosolids amendments were due to increases in the availability of nutrient to the plants. However, it would be reasonable to further examine the influence of biosolids, not only on the seed and biomass yields, but also on the various yield components of different crops under field conditions.

In Australia, compared to other grain crops canola needs about 25 % more N, P and K, than standard wheat to balance fertilizer inputs with nutrient removal in grain. In addition to this, the inclusion of canola under crop rotation provides additional benefits such as the control of cereal root diseases to a subsequent crop. Nevertheless, the responses and efficiency of canola in extracting nutrients (N, P, Cu and Zn) from land applied biosolids requires investigation, since gaining an understanding and knowledge in this area would probably enhance particularly for the wider use of canola on biosolids amended land.

2.9. Environmental risk assessment of biosolids

Among, all chemical contaminants, heavy metals are believed to be of a specific ecological, biological and/or health significance. Knox *et al*., (1999) refers to soil contamination as soil whose chemical state deviates from the normal composition but does not have a detrimental effect on organisms, whereas pollution occurs when an element or a substance is present in greater than natural (background) concentrations as a result of human activity and has a net detrimental effect on the environment and its components.

For comparison purposes the maximum permissible concentration limits of heavy metals for various countries including Australia is shown in Table 2.10, whereas Table 2.11 shows the ceiling limits for heavy metals for soils receiving biosolids under Victorian conditions.

Table 2.10 Maximum permissible concentration limits (μg/g) of the heavy metals Cd, Cu, Zn, Pb in agricultural soils in various countries and in the European Union

	Heavy metals				
Country	Cd	Cu	Zn	Pb	References
USa	20	750	150	140	McGrath *et al*. (1994)
Canada	0.5	30	25	50	IAEA (2004)
Australia	1	100	150	200	McLaughlin *et al.* (2000)
UKb	3	135	300	300	Johansson *et al*. (1997)
Germanyc	0.4-1.5	20-60	40-100	60-200	IAEA (2004)
Franceb	2	100	40	300	Johansson *et al*. (1997)
Netherlandsd	0.8	36	85	140	McLaughlin et al. (2000)
Norway	1	50	50	150	Haiyan & Stuanes (2003)
Swedenb	0.4	40	40	100(150)e	SNFS (2005)
Finland	0.3	32	38	90	IAEA (2004)
Denmark	0.3	30	40	100	IAEA (2004)
EUf	1-3	50-140	50-300	150-300	EC (1986)
China	0.3	50	250	200	SEPAC (1995)

a- Computed from maximum cumulative pollutant loading limits without taking into account background concentration of the elements in soils.
b- For soils intended to receive biosolids for crop production
c- the first and second values refer to sandy and clay soils respectively
d- Standard soil with 25 % clay and 10 % organic matter. Concentrations of elements are normalized based on clay (CL) and organic matter (M) content of soil.
(Cd=0.4 + 0.007X [CL+3M]; Cu=15 + 0.6X [CL+M]; Pb=50 + [CL+M] ;
Zn = 50 + 1.5X [2CL+M]).
f- For biosolids receiving soils with a pH of 6-7; e- figure in parenthesis refers to only some parts of Sweden

To determine the hazard posed by those heavy metals that do not increase in concentration as food chains rise, Heemsbergen *et al*. (2009) calculated a hazard quotient by dividing the maximum or the mean concentration of the heavy metals in a range of biosolids from each State participating in the NBRP by their corresponding soil limits (Ecological Investigation Levels- EILs) from the National Environment Protection (Assessment of Site Contamination) Measure (NEPC, 1999). Hence, based on their findings, Cd, Cu and Zn were selected as the three metals to be the focus of the NBRP. Table 2.12 shows the maximum and mean concentrations of metals in biosolids that do not bioaccumulate, their soil limits (EILs) and the ranking of the hazard they cause (Heemsbergen *et al*., 2009).

Table 2.11 The maximum and mean concentrations of metals in biosolids that do not bioaccumulate, their soil limit (EILs) and the ranking of the hazard in Australia.

Heavy metals	Maximum concentration in biosolids	Mean concentration in biosolids	Ecological investigation limits (EILs)	Ranking hazard based on maximum concentration	Ranking hazard based on mean concentration
Cu	1500	645	100	1	1
Pb	190	60	600	7	7
Mn	370	180	500	5	4
Ni	80	32	60	3	3
Zn	2000	740	200	2	2

The heavy metal with a ranking of 1 poses the greatest hazard and the heavy metal with a ranking of 7 poses the lowest hazard. Adapted from the NBRP, 2007

The NBRP carried out a preliminary hazard assessment for identified heavy metals in biosolids that caused the greatest hazard Cd, Cu and Zn and compared to those caused by the same metals in soluble salt form. This was conducted in order to establish the relative biological availability of the heavy metals from biosolids and from metal salts which would be utilized to derive the guidelines for metals in biosolids amended soils.

Grain Cd concentrations, toxicity of Cu and Zn to agricultural crop species and soil microbial processes (microbial respiration and nitrification) were used as end points. Critical soil concentrations of Cu and Zn that adversely affected plant productivity and microbial processes across all NBRP sites were determined. From their findings it was concluded that the recommended heavy metals limits largely depended on soil properties particularly pH, organic matter, clay content and cation exchange capacity. Soils with high soil pH, high organic carbon content, high clay content and high cation exchange capacity can receive considerable additions of biosolids and are considered to be at low risk from biosolids application and hence the recommended limits would noticeably be smaller to markedly larger than the current total limits of 100-200 μg/g for Cu and 200-250 μg/g total zinc. Conversely, soils with a combination of low pH, low organic carbon content, low clay content and low cation exchange capacities are considered to be at high risk from the application of biosolids.

Table 2.12, 2.13 and 2.14 show the maximum permitted total Cd and added Cu and Zn concentrations in soils receiving biosolids to prevent toxic effects to plants and soil microbial population (Heemsbergen *et al*., 2009).

Table 2.12 Recommended maximum permitted total cadmium (Cd) levels in soils receiving biosolids to ensure wheat grain does not exceed the Australian cadmium food standards (FSANZ, 2005).

Recommended maximum permitted total cadmium in soils receiving biosolids (µg Cd/g soil)			
pH	Clay content (%)		
	5	25	50
4.5	0.50	1.1	1.9
5.5	0.6	1.3	2.0
6.5	0.80	1.4	2.2
7.5	0.9	1.5	2.3
8.5	1.1	1.7	2.5

EPA Victorian maximum cadmium upper limits for classifying biosolids as grade C1 (contaminant grade 1) is 1 mg/kg on dry weight basis

Table 2.13 Recommended maximum permitted added copper (Cu) concentrations in soils receiving biosolids to prevent toxic effects to plants and micro-organisms

Recommended maximum permitted added Cu concentration in soils receiving biosolids (µg added Cu /g soil)			
pH	Organic carbon content (%)		
	1	2	5
5.0	25	50	135
5.5	40	75	175
6.5	90	185	200
7.5	205	230	230
8.0	245	245	245

EPA Victorian maximum cadmium upper limits for classifying biosolids as grade C1 (contaminant grade 1) is 100 mg/kg on dry weight basis

Table 2.14 Recommended maximum permitted added zinc (Zn) concentrations in soils receiving biosolids to prevent toxic effects to plants and micro-organisms.

Recommended maximum permitted added Zn concentration in soils receiving biosolids (µg added Zn /g soil)				
	Cation exchange capacity ($cmol_c$/kg)			
pH	3	10	20	60
4.5	20	45	75	165
5.5	38	90	145	300
6.5	70	165	265	300
7.5	130	300	300	300
8.0	180	300	300	300

The suggested total permitted zinc concentration is obtained by adding the ambient background concentration to the values presented.
EPA Victorian maximum cadmium upper limits for classifying biosolids as grade C1
(contaminant grade 1) is 200 mg/kg on dry weight basis

2.10. Economic valuation of biosolids

Roka *et al*. (2004) used the unit prices of commercially available products and multiplied them by the quantities of plant available nutrients and lime equivalents in the biosolids to compute the per-tonne value of biosolids. The economic value of biosolids nutrients N, P, K and dolomite lime in one tonne of the particular biosolids was estimated to be US$12.36, which not only depends on the nutrient composition of the material, but also on the application rate. The economic valuation of biosolids can be extended to include micronutrients since most biosolids contain micronutrients in a natural "chelated" form whereas commercially available chelated products can be expensive and hence the value of biosolids applications to agricultural cropland will be enhanced (Roka *et al*., 2004).

Several researchers have dealt with valuing the environment as an input in the production process. The theoretical aspects of these valuation techniques are described in (Freeman and Harrington 1990; Maler 1992 Freeman 1993 and Point 1994).

Maler (1991**)** distinguishes between applications of the production function approach. When production is measurable and either there is a market price for this output or one can be imputed, then the marginal value of the environmental good can be determined.

Case studies show the first year economic value of nutrients derived from biosolids in the Coastal Plains and Piedmont regions of Virginia range from US $25 to $50 per acre on pasture land and US $50 to $70 per acre on corn, small grains, and soybean land. The estimates were reported based on US $0.23 per pound for N, $0.30 per pound for P, and US $0.14 per pound for K (Faulkner, 2001).

2.11. Conclusions

The literature indicates that the effect of biosolids incorporation on the yields of various crops other than canola and oats has been extensively studied outside Australia. However, nutrient responses of crops and soil in biosolids amended soil under rotational cropping system have not been well documented.

The impact of crop rotation on N, P and heavy metals residues accumulated in plants and in biosolids amended soil and the leaching of nitrate-N needs to be further investigated. Also the effect of crop type on nutrient accumulation in biosolids amended soil has not been well documented.

Similarly, the relationship between crop yield and yield components due to biosolids nutrients needs further study.

A considerable level of investigation in US and UK has been conducted on the impacts of biosolids application on soil physical and chemical properties; however, the effect of biosolids application on pH and hence on heavy metals and phosphorus release and their relationship under field conditions deserve further attention.

Particularly under Australian conditions, the impact of biosolids loading rates on sulphur status of amended soil has not been studied; further research on sulphur may complement the previous biosolids land application work.

Sustainable utilization of nutrients from biosolids entails using nutrients efficiently and preventing off farm movement, and thus, there are significant information gaps surrounding ways to enhance the efficiency of utilizing nutrients from biosolids specifically under Australian soil type and environmental conditions (NBRP, 2007).

The effect of crop rotation on the quantity of nutrients and metals residue in biosolids amended soil has not been examined by the Victorian components of the NBRP works, certainly the benefits of land applications of various biosolids to farmers need to be evaluated on a crop rotation basis (Karen *et al.*, 1995) with due consideration given to the residual effects of plant nutrients and heavy metals on the succeeding crops (Binder *et al*., 2002).

Continuing research on the beneficial use of biosolids on agricultural lands needs to be conducted, because the build up of organic mater and accumulation of toxic heavy metals in amended soil are challenging to predict (Gaskin *et al*., 2003).

The levels of pathogens in biosolids amended soil after crop harvest could also be another area that needs to be evaluated under field condition.

Despite some studies conducted abroad on the economic value of biosolids from their nutrient perspectives, the economic value of biosolids as factor of input in the production process particularly using data generated from field and laboratory experiments has not been well documented for Australian soil type and environmental conditions.

The various experimental results conducted outside Australia can not be directly transferred to crops grown under Australian conditions, given the existing differences in soil and biosolids types, temperature, vegetation and crop management practices in Australia. Rather, site specific experiments with biosolids under Australian conditions using varieties of plant species need to be conducted so as to generate high quality data useful for sustainable utilization of biosolids by the end users.

A key emphasis will be given on quantifying the behaviour of anaerobically digested dewatered biosolids and composted biosolids applied on a clay loam soil at WWSP, Melton Victoria.

The next chapter describes in detail, the experimental design of a two year field trial conducted on a clay loam soil in Victoria Australia, using crop rotation involving canola and oats.

3

CHAPTER 3. MATERIALS AND METHODS

This chapter presents the laboratory and field experimental procedures employed during the period of the study; in particular it describes the soil and plant sampling and harvesting procedures and the details of the analytical techniques utilized to determine the various nutrients and heavy metals in biosolids amended soil and in plants.

The study was conducted on a clay loam soil on a 40×37 m^2 plot of land for a period of two years (2006-2007) at (WWSP) in Victoria. Canola and oats were grown under rotational cropping system. Dewatered biosolids and composted biosolids at various rates based on nitrogen limited biosolids application rates (NLBAR) were incorporated into the top 10 cm soil. The field experiment was arranged in a randomized blocked experimental design with six treatments each in triplicate including unamended and fertilized control plots. Crops were watered twice a week using a sprinkler watering system and were harvested after a period of six months.

Biosolids amended soil samples were taken after end of each year's experiment, whereas plant samples were taken at specific growth stages of the two crops for nutrients and heavy metals analysis.

3.1. Description of the experimental area

Melton shire is located in the western volcanic basalt plains of Victoria with a dry temperate climate zone that experiences significant winds and variation in temperatures; it is situated within the rain shadow of the Otway Ranges. Consequently annual rain fall is low and erratic averaging between 465-600 mm, with higher rainfall in the hilly northern part of the shire. The combination of spring rainfall, low spring frosts, winter chilling and low summer makes Melton a favourable environment for growing crops such as canola and oats. Higher rainfall in the north is suitable for forestry plantations, which currently exist in the northwest of the Shire. The average minimum temperatures are lower in the northern hills by about 7 ^{0}C, and higher to the southeast of the Shire by nearly 10 ^{0}C. The average annual maximum temperatures around Melton and the southeast of the Shire range from 18-20 ^{0}C. The original vegetation that dominated Melton's grassy plain and grassy woodlands were kangaroo and wallaby grasses.

The major farming practice in the area includes oats, barley, wheat and canola as winter crops, in some cases integrated with sheep farming using alfalfa. The soils in the Melton South district are typically red sodosol..

Microclimates exist locally which vary the temperature with terrain changes, such as in valleys or hillsides. The warm climate in central and southern area makes it suitable for hazelnuts, olives, pom fruit and lavender growth (Melton Environmental Atlas Draft, 2007).

The anaerobically digested dewatered biosolids used for these field experiments were sourced from WWSP. These biosolids were produced through anaerobic mesophilic digestion at 35 ºC for approximately 15-30 days prior to dewatering. The dewatered biosolids were subsequently dried on a site within the treatment plant for two years prior to use in these experiments. The composted biosolids were obtained from Pinegro (major supplier of agricultural and horticultural soil improvement products at Deer Park Victoria). The composted biosolids were produced from biosolids at the Sunbury Waste Water treatment centre which were blended with screened green waste from local council collections. Composting was conducted over a twelve week period according to (Australian Standards 4454, 2003) guidelenes in order to ensure elimination of pathogens from the composted product.

Table 3.1 summarizes the various waste water treatment stages and the outputs produced at WWSP.

Table 3.1 A summary of the main waste water treatment processes employed in the waste water treatment purification centre (WWSP).

Waste water treatment stages	**Out puts produced from waste water treatment**
Preliminary stage	
breaks up and screens any solids reducing them to a desirable maximum size, wastewater then passes through a chamber where grit is deposited	The grit is not considered a sewage sludge or biosolids and is taken away and used for landfill
Primary stage	
Wastewater enters two settling tanks where solids settle to the bottom or float to the top. Solid particles that settle to the bottom of the tank are raw sludge. Particles floating on the top of the tank will be collected and buried	Sewage sludge produced by primary waste water treatment, the water content can be reduced by dewatering or thickening
Secondary stage	
Wastewater moves into large aeration tanks and the settled waste water is mixed with bacteriologically active sludge. The thickened sludge mixture is then continually aerated to encourage the growth of microorganisms (bacteria) which breakdown the organic matter into simple materials such as water and carbon dioxide. Separation of sludge from waste water and sent to a digester where the bacteria are eventually broken down over a period of about 15 days	Sewage sludge produced by secondary wastewater treatment with low solids is usually harder to thicken and dewater than primary sludge. Biosolids can be produced through stabilization processes
Tertiary stage	
From the secondary tanks the wastewater is returned to the inlet works and then to the primary sedimentation tank and mixed with the primary sludge. The mixed sludge is then concentrated in a consolidation tank and sent to the digester where it is heated to encourage anaerobic biological activity. The remaining wastewater then makes its way to the irrigation area where it then makes it way through a series of lagoons. This process takes approximately 170 days.	From the storage lagoons the treated wastewater is used to irrigate nearby farmland. Any surplus water is filtered and returned through the drainage system to the storages. Digested sludge is withdrawn from the bottom of the digester in to the sludge holding tank to store it until it can be pumped into a tanker. Then it is disposed to a confined site. Since 2008 biosolids are put through a belt press and air dried

3.2. Experimental setup and biosolids treatments

An experimental site with a size of 40 m × 37 m^2 was set aside for conducting the biosolids field trial at WWSP .The site had no cropping or any fertilizer application history (including biosolids application) and it is situated within Western Water at Surbiton Park (Figure 3.1). A 3 m buffer zone was established along the northern and western edges of the site to avoid the shading effect from the trees.

Soil and biosolids samples were taken before sowing the crops and physicochemical properties of the soil and biosolids were determined.

Anaeobically digested dewatered biosolids from WWSP and composted biosolids obtained from Pinegro (a compost supplier company based at Deer Park). Pinegro obtains its biosolids from Western Water (Waste Water treatment centre, at Sunbury) for composting purposes.

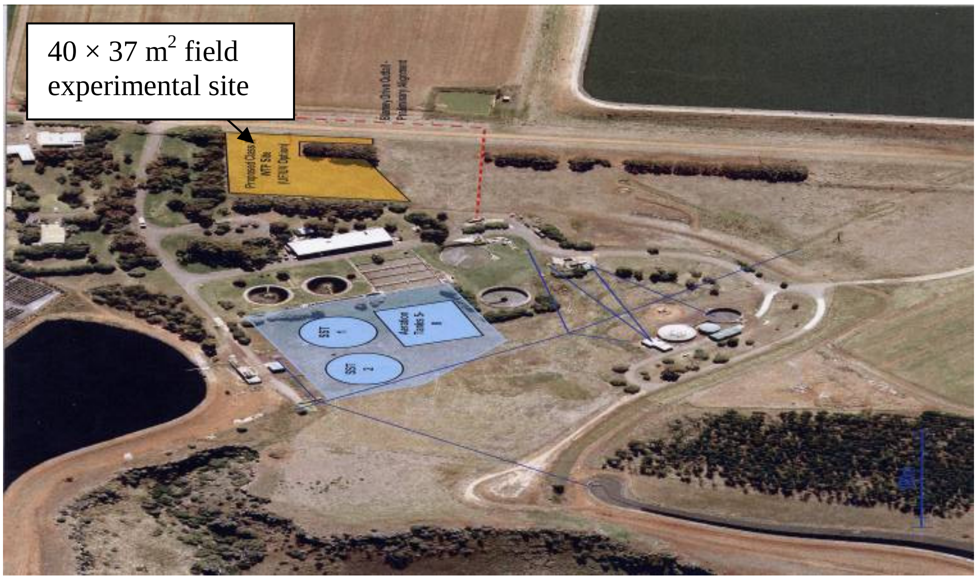
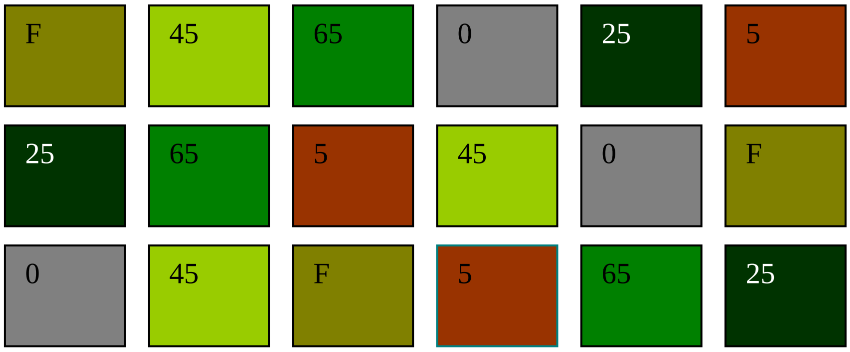
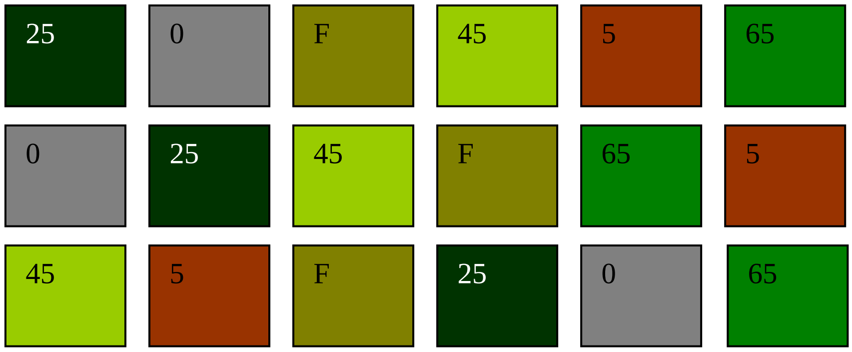


Figure 3.1 Arial map of the field experimental site at Western water, Surbiton Park, Melton South (Yellow shaded area indicated by the arrow).

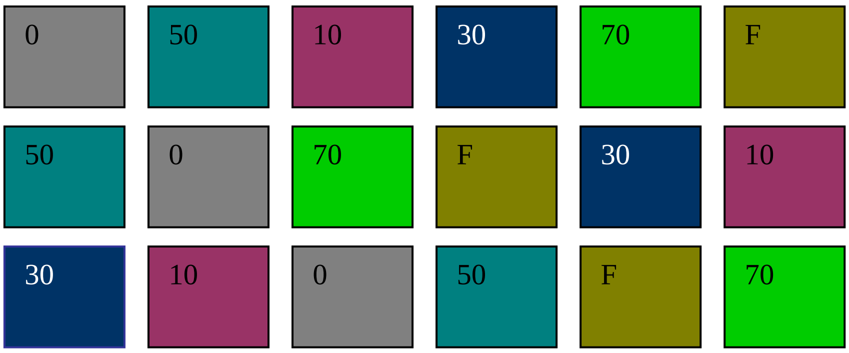
The dewatered biosolids were crushed using a front end loader. The experimental site was ploughed using first a tractor and then a rotary hoe. Gypsum ($CaSO_4$. $2H_2O$) was applied at 3 t/ha to inhibit dispersion of the soil aggregates and improve the soil structure and drainage. The land was partitioned into 72 experimental plots each 3 m wide and 4 m long (Figure 3.2 and 3.3).



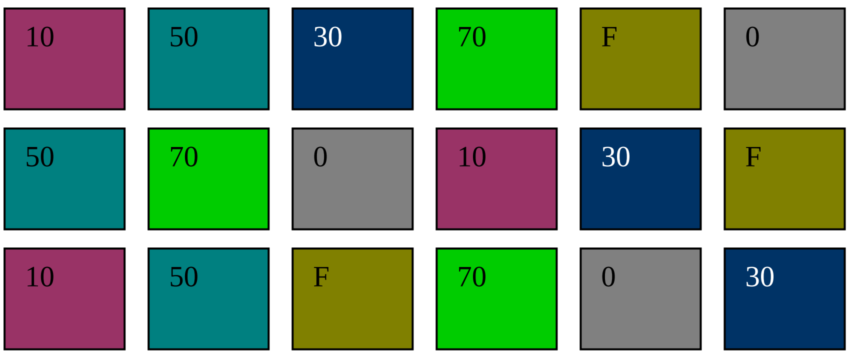
Canola plots treated with dewatered biosolids (Experiment 1)



Oats plots treated with dewatered biosolids (Experiment 3)



Canola plots treated with composted biosolids (Experiment 2)



Oats plots treated with composted biosolids (Experiment 4)

Figure 3.2 The randomized complete block design of the field experiments at Western water, Surbiton Park. The numbers 5, 25, 45, 65 t ds/ha refer to dewatered biosolids treatments, whereas 10, 30, 50 and 70 t ds/ha refer to composted biosolids treatments (application rates) respectively, while, C and F stands for unamended and conventionally fertilized control plots, respectively

Figure 3.3 shows land preparation and partitioning of the land into randomized block experimental design, and spreading and incorporation of biosolids into 10 cm soil depth

The land was partitioned into 72 replicated plots each with a 12 m^2 area arranged in a randomized complete block design. All biosolids applications were calculated on a dry weight basis. The site consisted of four dewatered biosolids applications (5, 25, 45 and 65 t ds/ha) and four composted biosolids applications (10, 30, 50 and 70 t ds/ha) including unamended and conventionally fertilized control plots. The nitrogen limited biosolids application rates (NLBAR) refers to the maximum rate at which biosolids can be applied with out exceeding the agronomic nutrient requirements for the two crops, and were also calculated for each of the biosolids and crop type using the method recommended by NSW EPA, (1997) (Appendix A).

$$NLBAR(t/ha) = \frac{CNR(kg/ha)}{ABN(kg/t)}$$

ABN (kg/t) = (NH_4-N) + (Oxidisable-N) + (Organic-N ×MR)

where CNR, ABN and MR refer to crop nitrogen requirement and available biosolids nitrogen and mineralization rate, respectively.

As the major objective of this study was not determining the mineralization rates of biosolids, in calculating the NLBAR, 15% and 10% organic nitrogen mineralization rates were assumed for the anaerobically digested dewatered biosolids and composted biosolids respectively, based on values cited in ‘Guidelines for Environmental Management, Biosolids land application’ (EPA, Vic. 2004). Such assumptions might under or over estimate the nitrogen mineralization rates of each of the biosolids and it did not take into account the site specific characteristics (soil type, compositional behaviour of the biosolids matrix, moisture and other relevant environmental parameters). It is also assumed that 6 and 5% of the organic-N mineralization rates from first year application may continue in the second year for anaerobically digested dewatered biosolids and composted biosolids respectively.

One NLBAR was equivalent to 10 and 20 t ds/ha dewatered biosolids and composted biosolids, respectively for the canola crop. Similarly one NLBAR was the same as 11 and 22 t ds/ha dewatered and composted biosolids for the oat crop. The biosolids were incorporated in the top 10 cm soil depth using a rotary hoe.

The application rates and their NLBAR equivalents are shown in Tables 3.2 and 3.3.

Conventional fertilizers, urea (46% nitrogen) applied at a rate of 100 and 110 kg/ha for canola and oats, respectively in 2006, whereas in 2007, urea (46% nitrogen), super phosphate (41 % P_2O_5) and sulphate of potash (41% potassium) at rate of 100:20:50 and 110:20:50 kg/ha were applied to canola and oat as control plots, respectively.

Four experiments:- canola with dewatered biosolids, canola with composted biosolids, oats with dewatered biosolids and oats with composted biosolids- were performed in triplicate in a randomized complete block design (Fig 3.2). The field trial was conducted on two crops: canola (*Brassica napus*, cultivar Beacon) and oat (*Avena sativa*, cultivar Echidna). Canola and oats seeds were sown at a seeding rate of 5 and 100 kg/ha, respectively.

The sprinkler watering system was installed to be used in case the crop experience water scarcity in time of drought. The influence of irrigation on crop performance and nutrient interactions was not systematically included in the experiment and not investigated.

After the completion of the first field experiment in 2006, the second experiment was conducted in 2007 by rotating the canola and oat crops and reapplying the same rate of dewatered biosolids and composted biosolids as in 2006 using the same experimental design.

Table 3.2 Application rates and NLBAR equivalents for dewatered biosolids applied for canola and oats in the 2006 and 2007 field trials

Dewatered biosolids rates (t ds/ha),	NLBAR, 2006	kg N/ha, 2006	Org.-N expected to mineralize in 2007 from 2006 application (6%) kgN/ha	Dewatered biosolids rates (t/ha), 2007	Total amount applied in 2007 plus residue from 2006 Kg N/ha
Canola plots					
5	0.5	211	11	5	222
25	2.5	1055	56	25	1111
45	4.5	1899	102	45	2001
65	6.5	2743	147	65	2890
Oat plots					
5	0.45	211	11	5	222
25	2.3	1055	56	25	1111
45	4.0	1899	102	45	2001
65	6.0	2743	147	65	2890

The biosolids had 42.2 kg total N/tonne of dry dewatered biosolids and one NLBAR was equivalent to 10 t/ha and 11t/ha for canola and oats respectively; mineralization rates of 15 % and 6 % in the first and second year of the experiment were taken into account during calculations of NLBAR for 2006 and 2007 respectively.

Table 3.3 Application rates and NLBAR equivalents for composted biosolids applied for canola and oats in the 2006 and 2007 field trials

Composted biosolids rates (t ds/ha)	NLBAR, 2006	Kg N/ha, 2006	Org.-N expected to mineralize in 2007 from 2006 application (5%) kgN/ha	Composted biosolids rates (t/ha), 2007	Total amount applied in 2007 plus residue from 2006 Kg N/ha
Canola plots					
10	0.5	144	5	10	149
30	1.5	432	16	30	448
50	2.5	720	26	50	746
70	3.5	1008	36	70	1044
Oat plots					
10	0.45	144	5	10	149
30	1.4	432	16	30	448
50	2.3	720	26	50	746
70	3.2	1008	36	70	1044

Note: The biosolids had 14.4 kg total N/tonne of composted biosolids and one NLBAR was equivalent to 20 t ds/ha and 22 t ds/ha for canola and oats respectively; mineralization rates of 10 and 5 % in the first and second year of the experiment were taken into account during calculation of NLBAR in 2006 and 2007 respectively.

3.3. Sampling Procedures

3.3.1. Soil and biosolids sampling

Before establishing the field experiment at WWSP, nine composite soil samples at 10 cm soil depth from the site, dewatered biosolids from WWSP and composted biosolids samples from Pinegro were sampled, crushed, air dried for two weeks, sieved to 2 mm and stored for further analysis according to sampling and sample preparation procedures described under method 1A1 and 1B1 of Rayment and Higginson (1992). Analytical results for the soils and biosolids used throughout this study are provided in Table 4.1. Both biosolids were analysed only at the beginning of year one as they are known to be consistent in chemical properties on the basis of regular analysis (Personal communication).

After the end of the first and second year trials, 30 soil core samples per plot were taken to a soil depth of 10 cm using an auger (9 cm diameter). The 30 cores from each plot were combined into a single composite sample, providing a total of 72 samples. Samples were also crushed, air dried for two weeks and sieved to 2 mm and stored for further analysis. Sub samples were taken and ground to pass < 64 µm for analysis of total nitrogen and total carbon.

3.3.2. Plant sampling

Young matured canola seedlings were sampled at 4 - 5 leaf stage (days after sowing). The oat crops were sampled at 5-6 tillering (days after sowing) stage. Sampling was conducted randomly to provide sixty canola plants per plot bulked together one composite sample; this provided 2160 individual plants for analysis of total nitrogen (TN), NO_3-N, extractable-P and heavy metals. A total of 720 oats individual plants were sampled from dewatered biosolids and composted biosolids treated oats plots. Twenty oat plants per plot were sampled at random and bulked together as one sample which provided a total of 720 oats individual plants.

Samples were washed with deionised water and dried in an oven at 60 ^{0}C for three days. Dried samples were crushed and ground into particles using a ring mill and sub samples were taken and refrigerated at 4 ^{0}C prior to analysis.

3.4. Measurement of soil moisture, pH and electrical conductivity

The moisture content of soil and biosolids samples was determined according to Method 2A1 of Rayment and Higginson (1992) by drying 10 g of soil and biosolids samples (< 2 mm) at 105 ^{0}C to constant weight.

The pH_w and pH_{ca} of soil and biosolids samples were measured as per Method 4A1 and 4B2 (Rayment and Higginson, 1992) respectively using a 1:5 soil/water suspension. A 20 g air-dried soil sample (< 2 mm) was weighed in a plastic bottle to which 100 mL of ultra-pure water was added and mechanically shaken at 130 rpm using a Platform Mixer in a closed system for 1 hr and allowed to settle for 30 minutes. The pH meter (pH 211 Microprocessor pH meter) was calibrated and standardized using pH_4 and pH_7 buffer solutions. After measuring pH_w, 1 mL of 1 M $CaCl_2$ was added to each plastic bottle, shaken for a further 30 minutes and allowed to settle for half an hour. Then pH ($CaCl_2$) was determined.

Electrical conductivity of soil and biosolids were measured as per method 3A1 (Rayment and Higginson, 1992) using a 1:5 soil /water extract. A 20 g air-dried soil sample (< 2 mm) was weighed in a plastic bottle to which 100 mL of ultra-pure water was added and mechanically shaken in a closed system for 1 hr to dissolve soluble salts and allowed to settle for 30 minutes. The ECONSCAN conductivity meter was calibrated using 0.01 M KCl at 20 ^{0}C as a reference solution and results were reported as EC (µS/cm) at 20 ^{0}C on an air-dry basis.

3.5. Determination of exchangeable bases and cation exchange capacity

The major cations in soil and biosolids samples were determined using 1 M NH_4Cl as an extracting solution according to Method 15A1 of Rayment and Higginson (1992). An aliquot of 53.5 g analytical grade NH_4Cl was dissolved in 900 mL Milli Q water and the pH was adjusted to 7.0 by adding 5 - 6 mL of NH_4OH and diluted to one litre with ultra pure water (Milli-Q water) and stored in borosilicate glass.

A 5 g sample of air dried and sieved (< 2 mm) soil and biosolids, each in triplicate, were weighed in 250 mL plastic bottles and then 100 mL NH_4Cl extracting solution was added and shaken for 1 hr and centrifuged at 3000 rpm for 10 minutes and filtered using a Whatman number 40 filter paper and 75 mm plastic funnels.

A 5 mL aliquot of the filtered extract was taken and placed in a 50 mL volumetric flask to which 1 mL of 70 % HNO_3 (Analytical Grade) and 0.5 mL of praseodymium internal standard were added and diluted to the mark with 2 % HNO_3. The same treatment was applied to standards and blanks. Analytical grade ICP-MS multi element stock solution was used to prepare working standards. Standards, samples and blanks were analysed using an Agilent Technologies Model 4500 series 300 ICP-MS with HP ChemStation software with a detection limits for the quadrupole of 1–10 ngL^{-1} and for magnetic sector 0.01–0.1 ngL^{-1}.
The cation exchange capacity (CEC) was calculated as the ratio of milli-equivalent of total exchangeable bases (Na, Mg, Ca, K) to percent base saturation as per method 15B1 (Rayment and Higginson, 1992).

3.6. Determination of the extractable forms of heavy metals in soil and biosolids

The measurement involves equilibration of air-dried soil for two hours with an extraction solution at a ratio of 1:2. The extraction solution was 0.005 M with respect to DTPA, 0.01 M with respect to $CaCl_2$ and 0.1 M with respect to triethanolamine (TEA). The TEA helps buffer the extraction solution close to pH 7.3 and thus restricts dissolution of metals from soil at high pH. A pH 7.3 also favours formation of the Zn-DTPA complex (Lindsay and Norvell, 1969b) whereas $CaCl_2$ acts to suppress carbonate (CO_3^{2-}) solubility in calcareous soils. For one litre of extraction solution 1.97 g diethylene triamine penta acetic acid (DTPA, mol. Wt. 393.4), 1.47 g $CaCl_2$ dihydrate ($CaCl_2.2H_2O$) and 14.92 g triethanolamine $(CH_2CH_2OH)_3$ were separately dissolved in ultra-pure water and combined, 6.8 g of 35 % w/w HCl was added and diluted to about 990 mL with ultra-pure water and the pH was adjusted to 7.3 by adding 3 M HCl drop by drop. At the desired pH the solution was diluted to 1 litre with ultra-pure water following Rayment and Higginson (1992) Method 12A1 and stored in a polyethylene container in a cool place.
A 10 g aliquot of air-dried soil and biosolids samples (sieved < 2 mm) were weighed into a plastic bottle and 20 mL of the extraction solution was added and shaken for 2 hr, centrifuged at 3000 rpm for 5 minutes and filtered with a Whatman number 42 (11 cm) filter paper. Analytical grade multi element mixed ICP-MS stock solution containing Mn, Al, Fe, Cu, Cr, Cd, Co, Mo, Ni, Pb and Zn at a concentration of 100 mgL^{-1} was purchased from a commercial supplier (Graham B. Jackson). An intermediate stock solution was prepared from the mixed stock standards at a concentration of 1 mgL^{-1} for each micronutrient. Calibration standards were prepared from the intermediate multi-stock solutions. Then 0.2-1

mL aliquots of soil and biosolid extracts were pipetted into 50 mL volumetric flasks and 0.5 mL (5000 ppb) Praseodymium internal standard was added to standards, samples and blanks to check for matrix effect and instrument drift. The calibration standards, samples and blanks were diluted with 2 % nitric acid solution and analysed using ICP-MS as previously described under section 3.5.

3.7. HNO_3/H_2O_2 extractable heavy metals in soil, biosolids and plant matter

The concentrations of metals in soil, biosolids and plant matter were determined according to the procedures described by Benton (2001) by weighing 0.5 g samples into 5 mL of 70 % nitric acid in 150 mL Pyrex tubes and predigested at room temperature for 30 minutes. Samples were digested in an aluminium block at 125 ^{0}C for 1 hr and then 3 mL of 30 % hydrogen peroxide was added to each tube. After 1hr of digestion, the Pyrex tubes were removed from the digestion block and allowed to cool. A 2 mL aliquot of 30 % hydrogen peroxide was added to each of the samples and further digested at 125 ^{0}C for 2 hr. After a total of 4 hr digestion, samples were removed from the digestion block and allowed to cool to room temperature. The Pyrex tubes were rinsed with 2 % nitric acid and quantitatively transferred to 50 mL volumetric flasks using < 0.45µm pore (Whatman No. 42) filter paper and diluted to the mark with 2 % nitric acid. For the analysis, 0.5 mL of the extract was added in to 50 mL volumetric flask and diluted with 2 % nitric acid. Calibration standards were prepared from a multi element stock analytical grade solutions. Samples, standards and blanks were spiked with 0.5 mL (5000 ppb) praseodymium internal standard to check for matrix effect and instrument drift. Samples, standards and blanks were diluted to the mark using 2 % nitric acid and the heavy metals (Cu, Cd, Co, Cr, Mo, Mn, Fe, Pb, Ni, Zn) and major cations (Na, K, Ca, Mg, Al) were analysed using an Agilent Technologies Model 4500 series 300 ICP-MS with HP ChemStation software.

To validate the laboratory generated analytical data for soil and biosolids and plant samples ,certified reference materials CRM 031-040 (sewage sludge) and standard reference material SRM1573a (Tomato leaves) were analysed respectively, along with the samples, and the percentage recovery of each analytes in the standard reference material was calculated.

To check the reproducibility of the analytical data, five replicates of soil and biosolids samples were analysed and the coefficient of variation was determined.

3.8. Determination of total metals, total P and total S in soil and biosolids using XRF

Total metals in soil, biosolids and biosolids amended soil samples were analysed using a Bruker S4 pioneer (Bruker AXS, Karlsruche, West Germany) Wave Length Dispersion- X-Ray Fluorescence Spectrometry, equipped with LiF, LiF (200), Ge, PET, OVO -55 crystals with a detection limit ranging between 10-100 $\mu g g^{-1}$ for soil (Schlotz and Uhlig, 2002).

X-ray fluorescence analysis was carried out by weighing 8 g of each of soil, biosolids and biosolids amended soil samples and 2 multimixes XRF pelleting tablets each weighing 1 g were added in each of the samples and ground using a Zirconium made Lab Technics Ring Mill. The fine particulate samples were transferred into an aluminum cup and packed using an Enerpac hydraulic pressure packer. The pressed pellets were analysed in triplicate.

For XRF analysis of total metals in soil/biosolids and biosolids amended soils samples, external calibration curves for each of the metals ((Cu, Cd, Co, Cr, Mo, Mn, Fe, Pb, Ni, Zn,) and major cations (Na, K, Ca, Mg, Al) were established by analysing eight soil standard reference materials (NCS DC 73319, NCS DC 73320, NCS DC 73321, NCS DC 73322, NCS DC 73323, NCSDC 73324 NCS DC 73325 NCS DC 73326). To validate the established calibration curves, Till 1 and Till 3 soil standard reference materials were treated as samples and analysed based on the already established calibration curves and the percent recovery was calculated.

3.9. Determination of total nitrogen, NO_3-N and NH_4-N in soil, biosolids and plants

Total nitrogen and carbon in soil and total nitrogen in plant matter

In collaboration with the Department of Primary Industry (DPI Werribee, State Chemistry Laboratory), 0.5 g samples of each of soil, biosolids and ground canola and oats shoots were weighed and analysed in triplicate to determine the percent total nitrogen. The Leco FP 2000 Carbon and Nitrogen Autoanalyzer instrument was calibrated using EDTA, Australian Soil and Plant Analysis council (ASPAC) standard reference materials (STD 75, STD 55 and compost 5 for soil and biosolids) and ASPAC standard reference materials STD 143 tea leaves and STD 63 eucalyptus leaves were also analysed for total nitrogen concentrations to validate the accuracy of analytical results. Total nitrogen in canola seed was determined following the Kjeldahl procedure.

NO_3-N in soil, biosolids and in plant matter

Nitrate-N was determined by an automated spectrophotometric method (flow injection analysis). In this method nitrates are reduced to nitrite by a copper cadmium reductor coil (CRC). The nitrite ion reacts with sulfanilamide under acidic conditions to form a diazo compound. This couples with N-1-naphthyl ethylenediamine dihydrochloride to form a reddish purple azo dye (Technicon Instrument Corporation, 1971).

Nitrate-N in samples of soil and biosolids were extracted using 2 M KCl extracting solution (Keeny and Nelson, 1982) which was prepared by dissolving 149.1 g of KCl in 1 L of milli-Q water. Five gram of soil and biosolids were weighed into 250 mL plastic bottles and 50 mL of 2 M KCl extraction solution was added and shaken in a Griffen Flask shaker for 30 minutes, and filtered using a Whatman number 42 filter paper (11 cm) and stored in a cool (4 ^{0}C) place. For the analysis of nitrate in plant tissue, 0.2 g of ground plant was weighed and 50 mL of 2 % acetic acid was added and shaken for 30 minutes, filtered using Whatman No 42 filter paper (11 cm) and stored in a cool (4 ^{0}C) place (Karla, 1998).

Samples, standards, and blanks were analysed using the cadmium-reduction technique in a flow injection analyser with the absorbance of the reddish purple colour measured at a wavelength 520 nm. Where concentrations exceeded the calibration range, soil biosolids and plant extracts were diluted with the extraction solutions in a 1:50 dilution factor and analysed.

The portable flow injection analyser instrument which was manufactured by Monash University was for the first time used in this study for the analysis of nitrate and phosphorus in soil and plant extracts. Figure 3.4 shows the instrument setup and the direction of flow of reagents, carriers and buffer solutions for the analysis of NO_3-N in soil and plant extracts.

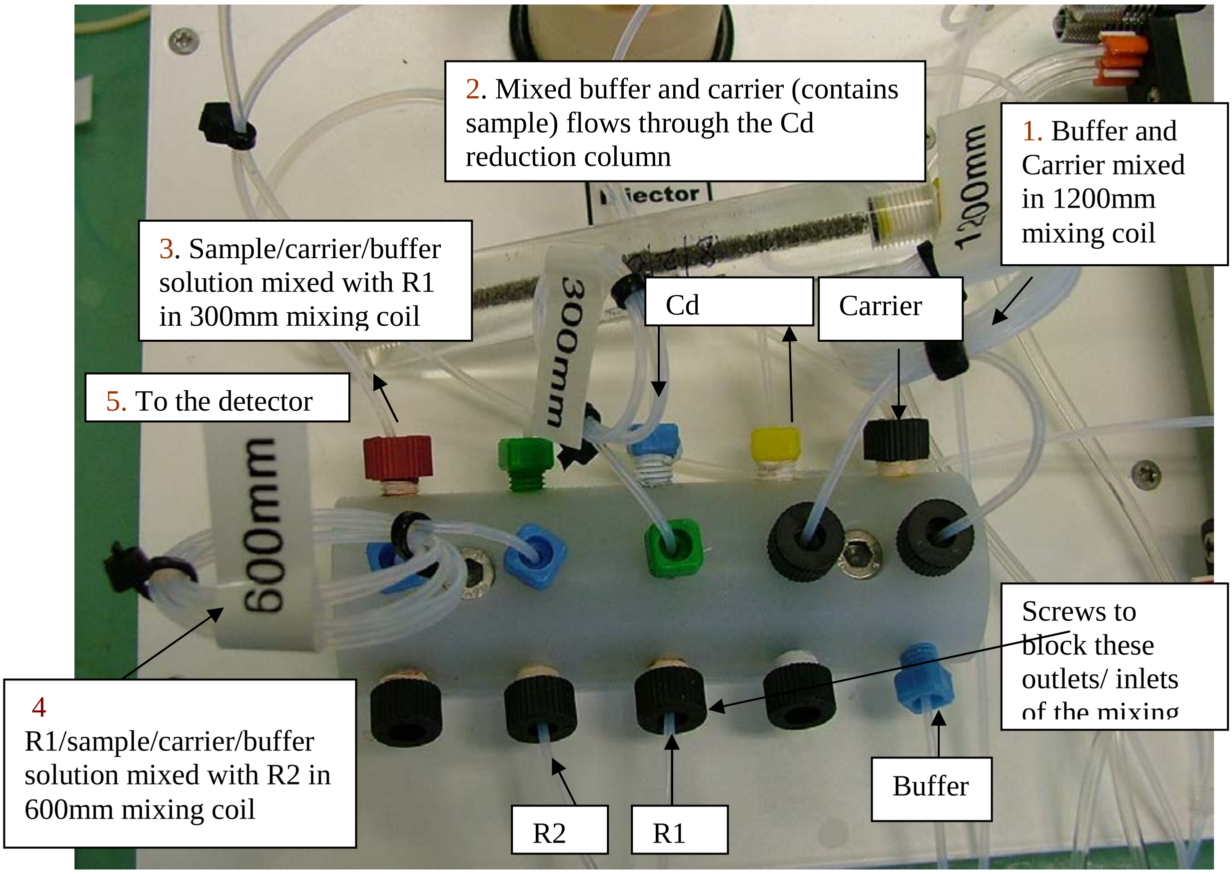


Figure 3.4 View of the mixing block setup for nitrite/ nitrate analysis. The numbers refer to the order wherein the reagents are mixed and show the direction of flow, R1 and R2 indicate reagent1 and reagent2

The following reagents, standard and buffer solutions were prepared and used:

Reagent 1 Sulphanilamide ($C_6H_8N_2O$)

Five gram of sulphanilamide was dissolved in a mixture of 26 mL concentrated HCl and 300 mL of ultra pure water added to a 500 mL volumetric flask. This was diluted to the mark with Milli-Q water and sonicated for 30 minutes.

Reagent 2 NED dihydrochloride solution ($C_{12}H_{14}N_2.HCl$)

Analytical grade NED dihydrochloride (0.5 g) was dissolved in 500 mL Milli-Q water and sonicated for 30 minutes.

Buffer NH_4Cl solution

To prepare the buffer solution, 85 g of NH_4Cl and 3 g of Na_2 EDTA were dissolved in 920 g of ultra pure water. The pH of the solution was adjusted to 8.5 by adding 5 - 6 mL concentrated ammonia; Milli-Q water was used as a carrier.

Preparation of copperized-cadmium reduction column

A 2% copper sulphate solution was prepared by dissolving 10 g $CuSO_4.5H_2O$ in 500 mL of ultra pure water.
Cadmium granules (10 g) were weighed and swirled in 6 M HCl for 1 min after which the supernatant was decanted to an appropriate waste container to remove the oxide coating from the cadmium granules. The cadmium granules were rinsed with ultra pure water and decanted. The cadmium granules were then swirled in portions of 2 % copper sulphate for 5 minutes and the supernatant was decanted. This process was repeated until a brown colloidal precipitate formed; finally this was washed with Milli-Q water and rinsed with NH_4Cl buffer solution. The granules sat in the buffer solution while packing the column.

Stock NO_3-N solution (100 mg/L NO_3-N)

Analytical grade sodium nitrate 0.3035 g ($NaNO_3$) was dissolved in 500 mL of ultra pure water. For the intermediate stock standard solution 20 mL of the 100 mgL^{-1} stock solution was diluted with Milli-Q water in a 100 mL volumetric flask.
Calibration standards were prepared by taking series of volumes (0, 1, 2, 4, and 8 mL) of the intermediate stock solution and diluted to 100 mL with Milli-Q water which were equivalent to 0, 0.2, 0.4, 0.8 and 1.6 mgL^{-1} NO_3-N (Appendix I Table I-1).

Validating the Analytical data

There are no standard reference samples for accurate determination of nitrate. Precision measurements for NO_3-N carried out for soil test quality assurance program of the Alberta Institute of Pedology (Heaney et al., 1988) indicated that NO_3-N was one of the most variable parameters measured. Coefficient of variation ranged from 4.8% to 30.4% for samples with 67.3 ± 3.2 (sd) and 3.3 ± 1.0 (sd) mg NO_3-N/ g respectively.

Hence, to validate the analytical data, extracts were spiked with 0.5 mL of the 20 mgL^{-1} NO_3-N standard and analysed. The percentage recovery was calculated.

NH_4-N in soil and biosolids (Phenate method)

Ammonium in soil and biosolids was determined by an automated spectrophotometric method (segmented flow analyzer) utilizing the Berthelot reaction (Searle, 1984). Phenol and NH_4 react to form an intense blue color. The intensity of the color is proportional to the NH_4 present. Sodium hypochlorite and sodium nitroprusside solutions are used as oxidant and catalyst, respectively (O.I. Analytical, 2001b).
Ammonium-N in soil and biosolids was analysed using the phenate method in which 4 g soil, biosolids were weighed in plastic containers in duplicate and 40 mL of 1 M KCl was added, shaken for 30 minutes, centrifuged, filtered and analysed using segmented flow analysis.

3.10. Determination of Olsen-extractable P in soil and biosolids

The empirical method of Olsen *et al.* (1954) has wide international acceptance as an indicator of soil P fertility. Olsen's method is based on extraction of air-dried soil with 0.5 M $NaHCO_3$ and adjusted to pH 8.5 with NaOH. Soil extraction is for 30 minutes at a soil/solution ratio of 1:20 as per Method 9C1 (Rayment and Higginson, 1992).

Extracting solution

A 0.5 M NaHCO3 extracting solution was prepared by dissolving 42 g sodium bicarbonate in 900 mL ultra pure water and 0.8 g NaOH was added to adjust the pH to 8.5 and diluted to 1000 mL with ultra-pure water.

Reagent 1 (Ammonium Molybdate)

For the first reagent, 5 g ammonium molybdate was transferred to a 500 mL volumetric flask, 250 mL of ultra-pure water was added and sonicated until completely dissolved, then 70 mL of 25 % v/v sulphuric acid added and diluted to the mark with ultra-pure water and sonicated for 15 minutes.

Reagent 2 (stannous chloride)

The second reagent (stannous chloride) was prepared by weighing 0.1 g tin (II) chloride and 1 g hydrazine sulphate in a 500 mL volumetric flask and 250 mL of ultra-pure water was added and sonicated for 30 minutes until both compounds dissolved, then 56 mL of 25 % sulphuric acid was added and diluted up to the mark with ultra-pure water and sonicated for 15 minutes.

Standard stock solution (100 mg P/L)

Phosphorus stock solution was prepared by dissolving 0.4394 g of KH_2PO_4 in ultra-pure water and made up to a final volume of 1000 mL in ultra-pure water.

Intermediate stock solution (20 mgP/L) and Calibration Standard solutions

For the intermediate stock solution, 20 mL of the 100 mg P/L stock solution was diluted with Milli- Q water in 100 mL volumetric flask and working calibration standard solutions were prepared by taking 1, 2, 3,4,5,7.5 and 10 mL of the intermediate stock solution in 100 mL volumetric flasks which were equivalent to 0.2, 0.4, 0.6, 0.8, 1, 1.5 and 2 mg P/L concentrations and diluted up to the mark with the extracting solution (Appendix I Table I-2).

Hence, 2.5 g of soil and biosolids samples were weighed in 125 mL plastic bottles to which 50 mL of the extracting solution was added, shaken in a reciprocating shaker for 30 minutes and filtered using Whatman number 42 filter paper (11cm).

The portable FIA system shown in Figure 3.5 was setup according to the manufacturer’s manual for P analysis in soil and plant extracts and the absorbance of the blue complex was recorded at 640 nm.

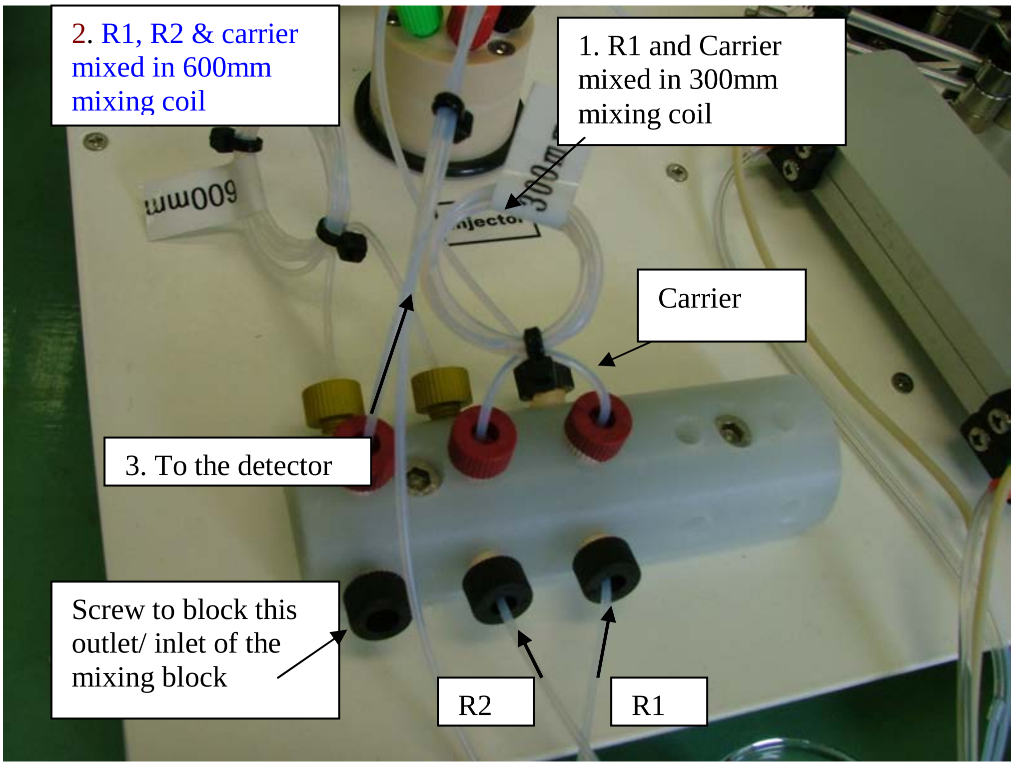


Figure 3.5 View of the mixing block setup for phosphorous analysis. The numbers refers to the order wherein the reagents are mixed and the arrows show the direction of flow

Soil and biosolids extracts were analysed in triplicate by diluting them with extraction solution in a 25 mL volumetric flask. Olsen and acetic acid extractable soil and plant phosphorus data were validated by spiking soil extracts with a known concentration (0.8 μgg^{-1}) of phosphorus standards and plant extracts with 0.8 and 2μgg^{-1} phosphorus standards and analysed. The percent recoveries of the spiked samples were calculated.

To further check the quality of Olsen- extractable phosphorus data, an ASPAC 51 soil standard reference material was also treated as a sample and analysed for phosphorus concentrations to determine the percent recovery.

3.11. Acetic acid extractable phosphorus in plant matter

For the determination of phosphorus in plant matter, 0.2 g of ground plant samples were weighed in 125 mL plastic bottle in triplicate and 50 mL of 2 % acetic acid was added, shaken for 30 minute and filtered using a Whatman number 42 filter paper (Karla, 1998). The phosphorus concentration in plant extracts was determined using the stannous chloride reduction technique in the flow injection analyser.

To validate the quality of analytical data, plant extracts were spiked with a known concentration of phosphorus standards ranging between 0.8 and 2 μg/g and the percentage recovery was calculated.

3.12. Pathogen survival in biosolids and biosolids amended soil

At the end of the 2006 trial, random samples of 20 cores of biosolids amended soil per plot at 0-10 cm soil depth including from unamended control plots were taken from experiment 1 (canola with dewatered biosolids treated plots), and combined to make a composite sample to give a total of 18 composite samples. Dewatered biosolids and composted biosolids samples were also randomly taken. Similarly, at the end of 2007 trial, the same sampling procedure was followed. All biosolids amended soil and biosolids samples were submitted to the Australian Laboratory Services (ALS Consulchem) and analysed as per Australian Standards, (AS5013.11.1, 2004) for survival of the pathogens *E.coli*, *Clostridium perfringens* and *Salmonella species*.

E.coli/g was analysed as per AS5013.15, 2004, (37 ^{0}C/48h), *Clostridium perfringens*, cfu/g was analysed as per AS5013.16, 2004, (37 ^{0}C/20 h) and *Salmonella spp*, in 25 g as per F0677 (37 ^{0}C/16-20 hr). The analysis was performed on samples as received.

3.13. Harvesting crops and determination of yield and yield components

Canola

From each of the 36 canola experimental plots which includes experiment 1 (Canola with dewatered biosolids and Experiment 2 (Canola with composted biosolids) at WWSP, plant biomass samples from a 1 m^2 area in the centre of the plots to avoid edge effects were harvested. Sub-samples of 5-10 individual plants were taken and plant height, number of branches/plant, number of pods/plant and number of seeds/pod and 1000 seed weight were recorded.

Then samples were allowed to dry for 7 days and dry plant biomass was recorded, pods were crushed and seeds were separated from the pods and dry seed weight was taken.

Samples of canola seed were sent to Agrifood Technology at Werribee, Victoria and seed oil content was extracted using solvent extraction and the concentration of oil in canola seed was determined by gas chromatography.

Oats

From each of the 36 oats experimental plots treated with dewatered and composted biosolids (including the conventionally fertilized and unamended control plots), plant samples were harvested from a 1 m^2 area in the centre of each of the plots and allowed to dry for 7 days. The dry plant biomass and seed yields were recorded.

Separate sub-samples of 5-10 individual oat plants were also taken and measured for agronomic variables grain/panicle and plant height.

3.14. Statistical procedures

The analytical data were subjected to a two way analysis of variance (ANOVA, F-test) taking into account biosolids application rates and blocking effect for each experiments using Genstat Release 9 (Lawes Agricultural Trust, Rothamsted, and Harpenden, UK). Differences between means were compared by Fisher's least significance difference LSD (t-test) using a significance of $P \leq 0.05$ level. Pearson correlation coefficients were used to determine significance of correlations between various parameters. Correlations were declared significant at $P \leq 0.05$ and highly significant at $P \leq 0.01$. Simple regression analysis was also conducted to examine the impact of biosolids application rates on soil P levels and multiple regression analysis was also performed to test the effect of soil total metals concentrations and soil pH on plant shoot metal concentrations. The superscripts *, **,*** and ns refer to significant treatment effects in ANOVA F-test at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ and not significant, respectively.

4

CHAPTER 4. THE RESPONSE OF CANOLA AND OATS TO THE APPLICATION OF BIOSOLIDS

Introduction

This chapter measures and compares the effect of incorporating various rates of dewatered and composted biosolids on the yield and yield components of canola and oats and their relationship under a crop rotation regime. It also describes the effect of different rates of dewatered biosolids and composted biosolids on N, P, S and oil concentrations in canola seeds under field conditions.

4.1. Physicochemical properties of soil and biosolids

The anaerobically digested dewatered biosolids generated from WWSP had been stockpiled on a 10 hectare land over two years. As expected the moisture content of the biosolids was very low since the biosolids were exposed to wind and hot summer heat. It is also expected that significant proportions of the NH_4-N and NO_3-N may have lost through NH_3-N volatilization and leaching respectively. Air drying had no significant effect on the moisture content of the biosolids, however, for the purpose of standard analytical procedures the biosolids were air dried and analysed for physicochemical properties.

After correcting for moisture content which was very low, the dried biosolids were weighed and incorporated into 0-10 cm soil depth at higher application rates on dry weight basis.

The composted biosolids were transported from pinegro (composting firm at deer Park) and left for a month near the experimental site. The composted biosolids had similar moisture content with the dewatered biosolids obtained from WWSP. Both the dewatered biosolids and composted biosolids were mainly domestic origin with slight industry discharge.

As part of its routine activity, the staff at Western Water collect and send biosolids samples to Australian laboratory Services for nutrient and heavy metal analyses every three months; the results indicate that there is no significant difference between the various temporal analytical results for nutrients and heavy metals (data not shown).

During the second year of the experiment, the same dewatered biosolids and composted biosolids were used and incorporated into 0-10 cm soil depth on dry weight basis.

Results for selected physical and chemical properties of the soil type, dewatered biosolids and composted biosolids used in the field experiment are shown in Table 4.1 below.

The pH and dry matter content of soil and biosolids products were relatively similar; however, the anaerobically digested dewatered biosolids had higher concentrations of total C, N, S, Cu and Zn concentrations than the composted biosolids. Biosolids from Western Water at Sunbury Waste treatment centre are blended with screened green waste from local council collections and passed through a 12-week biosolids composting process that produces sufficient heat to destroy any pathogens to produce a composted biosolids product.

The EC and CEC values of composted biosolids were significantly higher than dewatered biosolids which could imply that long term repeated applications of the composted biosolids may elevate the levels of soluble salts in the soil. Composted biosolids had also higher concentrations of total P, Olsen-P, NO_3-N which would possibly increase phosphorus levels in the amended soil. The concentrations of major cations with the exception of aluminium were significantly higher in composted biosolids than the levels found in dewatered biosolids; this would be due to the addition of green wastes during the composting process. Compared with the biosolids generated by Victorian (Goulburn valley Water, North East Water, Dutson Downs Gippsland and Dutson Downs East Gippsland water) which were used by the NBRP, biosolids produced at WWSP had considerably higher total N, P, Cu, Zn and NH_4-N concentrations.

Table 4.1 Physicochemical properties of soil and biosolids used for the experiments

Analytes	Soil	Dewatered biosolids	Composted biosolids
pH_w	6.5	6.7	6.4
DM (%)	98	92	89
$pHCaCl_2$	5.5	6.2	6.1
EC (1:5)(μ S/cm)	67.4	1350	2704
CEC (meq/kg)	6.9	24	62
Total N %	0.17 ± 0.002	4.22 ± 0.01	1.44 ± 0.003
Total C %	2.04 ± 0.02	31 ± 0.1	13.85 ± 0.04
C/N	12.2	7.4	9.6
Total P (μg/g)	855 ± 3	15003 ± 4	21290 ± 566
Total S (μg/g)	239 ± 1	11380 ± 0.0001	5265 ± 6
Total Cu (μg/g)	17 ± 1	648 ± 1	210 ± 4
Total Zn (μg/g)	37 ± 2	1062 ± 1	813 ± 8
Total Mn (μg/g)	247 ± 2	213 ± 1	299 ± 6
Total Ni (μg/g)	23.3 ± 0.4	27.17 ± 0.01	21 ± 0.4
Total Pb (μg/g)	11.4 ± 0.2	28 ± 3	47 ± 1
Total Co (μg/g)	BDL	3.97 ± 0.14	BDL
Total Cr (μg/g)	BDL	-	15.3 ± 0.6
Total Cd (μg/g)	BDL[a]	BDL	BDL
Total K (%)	1.071±0.005	0.389±0.001	1.240 ±0.002
Total Fe (%)	4.53 ± 0.01	1.36 ± 0.01	2.5 ± 0.1
Olsen-P (μg/g)	14.8	691	762
NO_3-N (μg/g)	2.9	830	1864
NH_4-N(μg/g)	5 ± 3	3740 ± 74	2113 ± 8
Mineral-N	7.9	4570	3977
Organic-N	1692	37630	10423
1 M NH_4Cl extractable Major cations (μg/g)			
Na	76 ± 2	749 ± 59	1557 ± 50
Mg	264 ± 4	1200 ± 86	3564 ± 17
K	316 ± 11	735 ± 65	4546± 79
Ca	697 ± 13	1831 ± 118	2864 ± 51
Al	1.3 ± 0.2	144 ±31	11 ± 9

Total P, S, Cu, Zn and Mn were determined using WD-X-ray fluorescence spectrometry, whereas, Na, Mg, K, Ca and Al were analyzed using ICP-MS (n = 3). The superscript "a "indicates that total cadmium in soil and biosolids was extracted by HNO_3/H_2O_2 and analyzed by ICP-MS and Cd content was below detection limit(BDL) . All analytical results are expressed on air dry basis.

4.2. Responses of canola to the application of biosolids

Figure 4.1 shows the growth of canola at various stages during the seasons in 2006 and 2007 experiments at WWSP.

Dewatered biosolids and composted biosolids applications compared to the nil control plots significantly ($p < 0.05$) increased the seed and plant biomass yield and yield components of canola crop (Table 4.2).

Compared to the unamended and conventionally fertilized control plots, increasing trends for all the agronomic variables following biosolids application were observed (Fig. 4.2 and 4.3).

Figure 4.1 Canola crops grown using dewatered and composted biosolids at Western Water Surbiton Park, 2006 and 2007 field experiments.

Year 1 (2006)

Table 4.2 presents the results for all the agronomic variables measured for the canola crop at all application rates and Figure 4.2 and 4.3 show the main agronomic variables plotted against application rates.

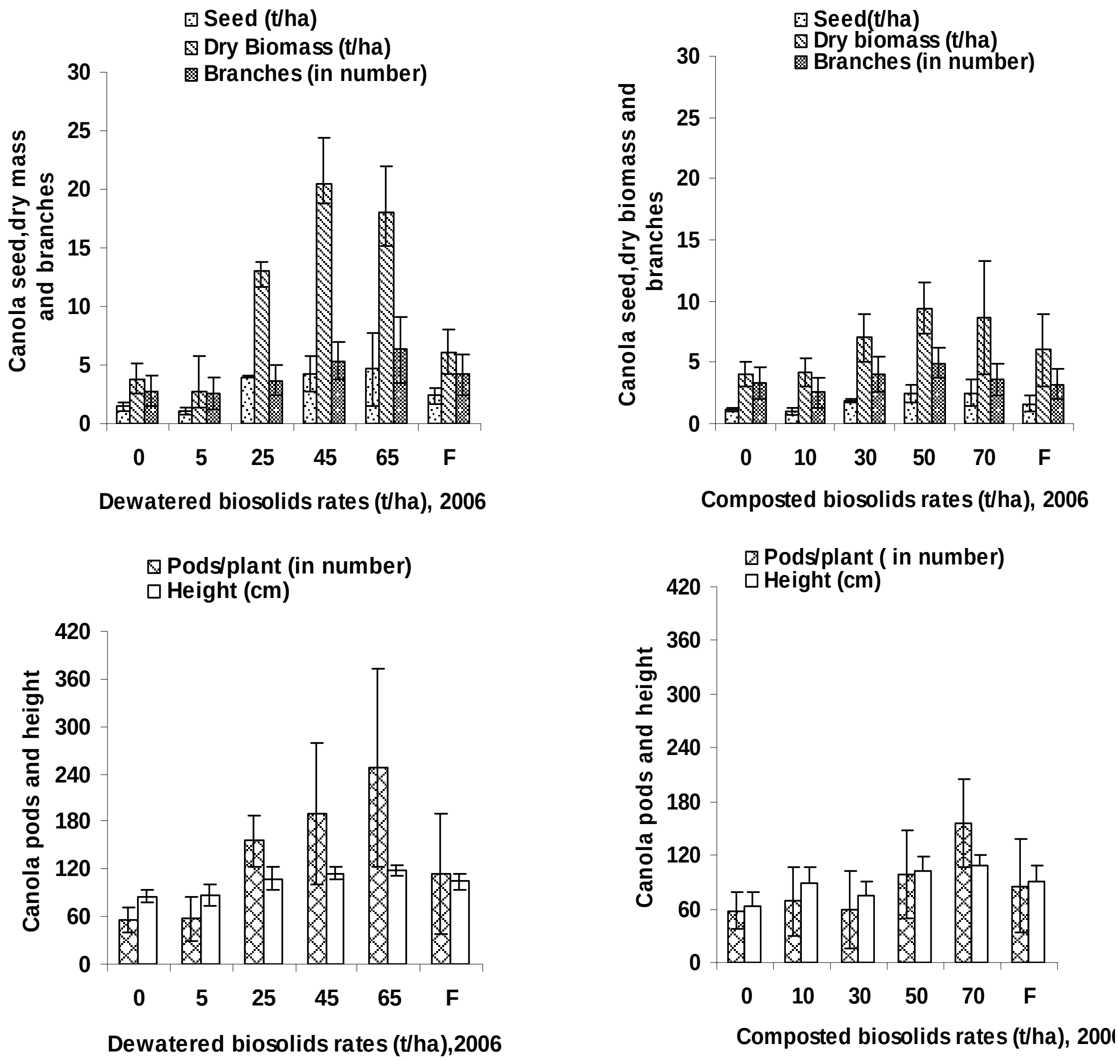


Figure 4.2 Canola seed and dry biomass yields and yield components as affected by dewatered biosolids and composted biosolids application rates on dry solid basis (2006).

Yields of canola seed and plant biomass recorded in the unamended control and the highest dewatered biosolids application (65 t/ha) treated plots were compared. Canola seed yield increased significantly from 1.4 to 4.6 t/ha, whereas plant biomass increased significantly from 3.8 to 20 t/ha, respectively ($p < 0.05$) (Table 4.2) and Fig 4.2 and 4.3).

Figure 4.2 depicts the mean values of canola yield and yield components as influenced by dewatered biosolids and composted biosolids applications, whereas, figure 4.3 shows the total raw data for canola seed and biomass yields plotted against biosolids applications.

The highest yields were attained at the highest application rate of dewatered biosolids (65 t/ha), however, canola seed yield recorded at 25, 45 and 65 t/ha dewatered biosolids application rates were not significantly different from each other, thus the 25 t/ha dewatered biosolids rates would be the optimum loading rate for the optimum canola seed production.

The number of branches per plant, pods per plant and plant height of canola crop were also highly significant compared with the unamended and fertilized control plots ($p < 0.001$) and responded positively as dewatered biosolids loading rates increased. The highest number of branches per plant (6), pods per plant (248) and plant height (118 cm) were recorded at the maximum dewatered biosolids application rate(65 t/ha) (Table 4.2).

Canola seed yield increased from 1.1 t/ha in the unamended control plot to 2.5 t/ha at the highest (70 t/ha) composted biosolids treated plot ($p < 0.05$). Similarly, canola seed yield obtained at the 30, 50 and 70 t/ha composted biosolids applications were not significantly different from each other and hence the optimum composted biosolids rate would be the 30 t/ha loading rate for the optimum canola seed yield.

Plant biomass yields obtained 9.3 t/ha at 50 t/ha application rate were significantly higher than the unamended and conventionally fertilized control plots. The highest number of branches per plant (5), pods per plant (156), and plant height (108 cm) of canola crop were recorded ($p < 0.001$) at the 50 t/ha composted biosolids application rate (Table 4.2).

In both dewatered biosolids and composted biosolids treated canola plots the number of seeds/pod and the weight of 1000 seeds were not different from the control plots.

Table 4.2 The response of canola to the application of various rates of dewatered biosolids and composted biosolids (t/ha) in the 2006 field experiment.

Experiment type	Applied rates (t/ha)	Dry seed Wt. (t/ha)	Dry biomass (t/ha)	Branches per/plant$_n$	Pods/plant$_a$	Seeds/pod$_n$	Height (cm)	1000 Seed wt. (g)
Dewatered biosolids treated canola plots	Control	1.4	3.8	3	56	25	86	3.4
	Fertilized	2.4	6.0	4	115	25	104	3.1
	5	1.0	2.8	3	58	24	86	3.4
	25	4.0[a]	13.0	4	156	26	108	3.4
	45	4.2	20.4	5	190	25	114	3.3
	65	4.6	18.0	6	248	26	118	3.4
	LSD $_{0.05}$	2.7*	4.9***	0.9***	47.8***	ns	8.6***	ns
Composted biosolids treated canola plots	Control	1.1	4.0	3.3	59	25	63	3.2
	Fertilized	1.6	6.0	3.2	68	25	88	3.4
	10	0.97	3.7	2.5	59	22	74	3.4
	30	1.9[b]	7.0	4.0	98	24	102	3.0
	50	2.4	9.3	5.0	156	22	108	3.1
	70	2.5	8.7	3.6	85	25	91	3.1
	LSD $_{0.05}$	1.1*	5.0*	0.63***	22***	ns	8.2***	ns

The superscripts *, ***, and ns refers to significant treatment effects in ANOVA at $p < 0.05$, $p < 0.001$, and not significant, respectively. The subscript "n" refers to branches, pods per plant and seeds per pod were expressed in number. The superscripts a and b indicate the optimum canola seed yields in response to dewatered biosolids and composted biosolids applications, respectively.

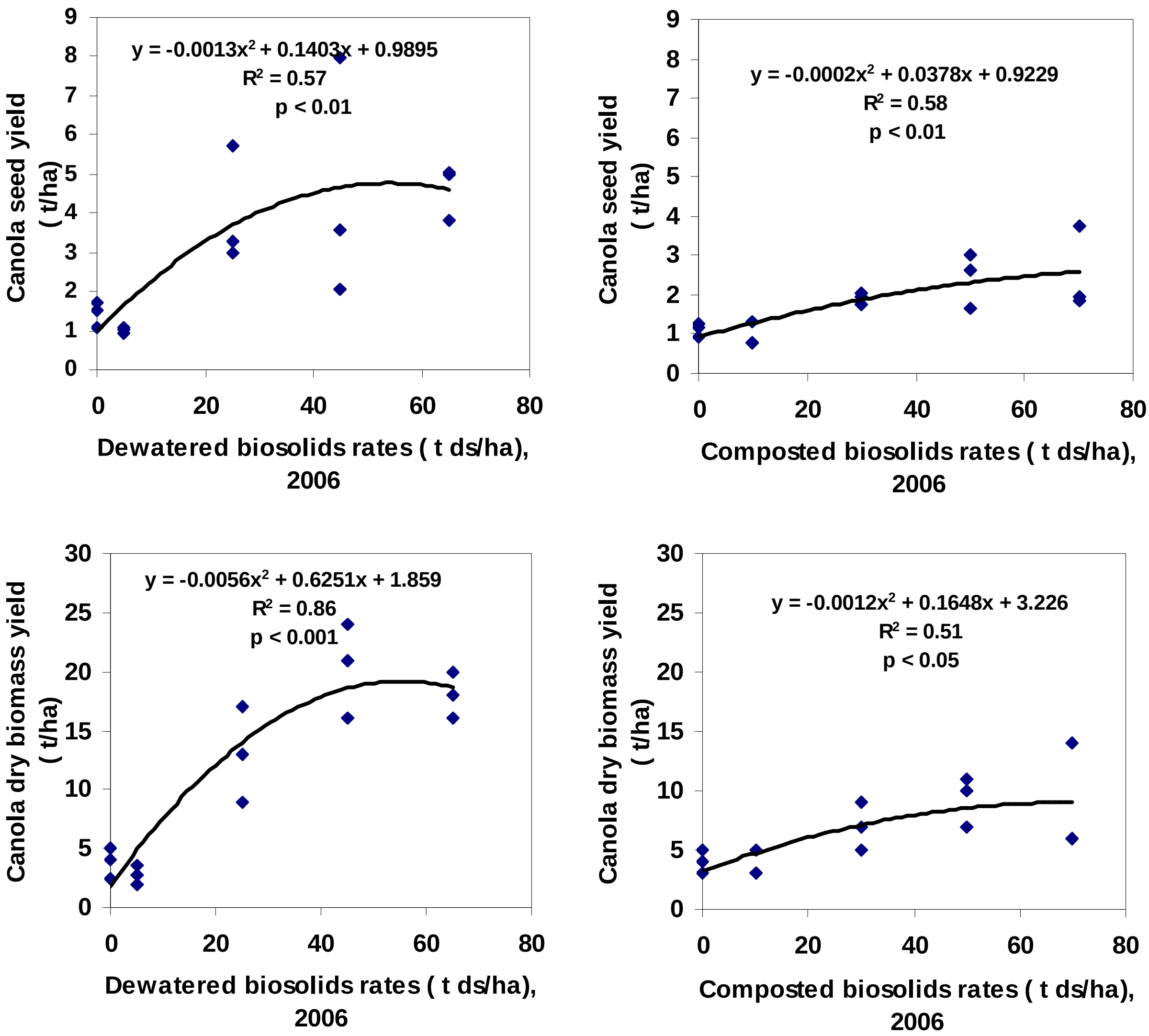


Figure 4.3 The effect of dewatered biosolids and composted biosolids applications on canola seed and dry biomass yields in the 2006 plot trial. Triplicate measurements for canola seed and dry biomass yields were plotted against biosolids application rates. The probability vaues stand for ANOVA (F-test) for the regression line.

Year 2 (2007)

Dewatered biosolids were reapplied at the same rates as in the 2006 experiment. Canola seed of 4.3 t/ha and plant biomass yields of 18 t/ha recorded at the 45 and 65 t/ha dewatered biosolids treated plots were significantly ($p < 0.001$) higher than both the yields recorded from conventionally fertilized and the untreated control plots (Table 4.3).

Similarly Figure 4.4 depicts the mean values of canola yield and yield components in response to dewatered biosolids and composted biosolids applications, whereas, figure 4.5 indicates the total raw data for canola seed and biomass yields plotted against biosolids applications.

Canola seed yields obtained from the 45 and 65 t/ha dewatered biosolids application were not significantly different from each other, thus the 45 t/ha dewatered biosolids application was the optimum dewatered biosolids application rate.

The number of branches (19), pods per plant (323) for canola were highest ($p < 0.001$) at the 45 t/ha dewatered biosolids loading rate, whereas the maximum number of seeds per pod (29) and plant height (120 cm) were recorded at the 65 t/ha dewatered biosolids loading rates ($p < 0.001$). Compared with the control plots, significant increases in seed and biomass yields, the number of pods/plant and branch/plant were observed ($p < 0.001$).

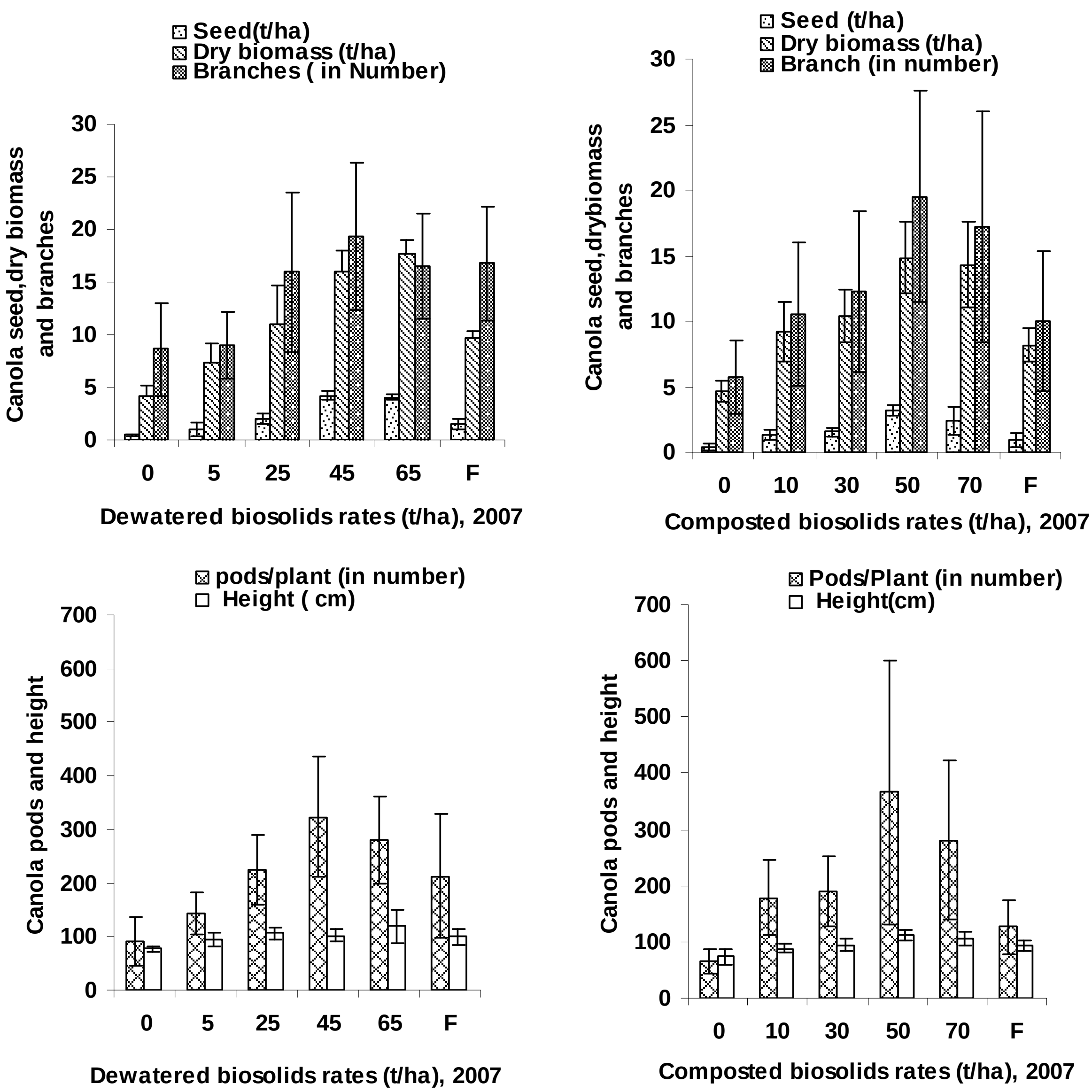


Figure 4.4 Canola seed and dry biomass yields and yield components as affected by dewatered biosolids and composted biosolids application rates on dry solids basis (2007). The error bars indicate ± sd of triplicate measurements.

Table 4.3 The response of canola to the application of various rates of dewatered and composted biosolids (t/ha) in the 2007 field experiment.

Experiment type	Applied rates (t/ha)	Dry seed Wt. (t/ha)	Dry biomass (t/ha)	Branches per/plant$_a$	Pods Per plant$_a$	Seeds per pod$_a$	Height (cm)	1000 Seed wt. (g)
Dewatered biosolids treated canola plots	Control	0.4	4.2	8.6	92	21	77	3.83
	Fertilized	1.5	9.7	17.0	213	26	99	4.08
	5	1.0	7.3	9.0	142	27	94	2.84
	25	2.0	11.0	16.0	224	26	107	3.4
	45	4.3^c	16.0	19.0	323	28	102	3.8
	65	4.0	18.0	16.5	280	29	120	2.8
	$LSD_{0.05}$	0.7***	4.5***	4.01***	54***	3.3***	12***	ns
Composted biosolids treated canola plots	Control	0.5	4.7	5.7	66	25	74	4.9
	Fertilized	1.0	8.2	9.9	127	28	93	4.9
	10	1.4	9.2	10	178	25	88	5.5
	30	1.6	10.4	12	189	28	95	5.6
	50	3.2^d	15.0	19	367	31	112	3.9
	70	2.4	14.3	17	281	29	106	3.6
	$LSD_{0.05}$	1.2**	4.3**	4.7***	85.7***	2.4***	7.6***	ns

The superscripts *, **, ***, and ns refers to significant treatment effects in ANOVA at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant, respectively, whereas the subscript 'a' refers to branches, pods per plant and seeds per pod were expressed in number. The superscripts 'c' and 'd' indicate the optimum canola seed yield for dewatered biosolids and composted biosolids, respectively.

Composted biosolids were reapplied at the same rate as in 2006 experiment and the highest canola seed and dry biomass yields of 3.2 and 15 t/ha were measured in the 50 t/ha composted biosolids loading rates. Likewise canola seed yields recorded at the 50 and 70 t/ha composted biosolids rates were not significantly different from each other suggesting the 50 t/ha composted biosolids rate was the optimum loading rate.

The number of branches per plant, pods per plant, seeds per pod and plant height was highest at the 50 t/ha composted biosolids treated plots which were significantly different from the unamended and conventionally fertilized control plots (Table 4.3).

The number of seeds/pod in canola was significantly different ($p < 0.001$) from the control plots for both dewatered biosolids and composted biosolids treated canola plots, however the weight of 1000 seed canola seed was not different from between the treatments.

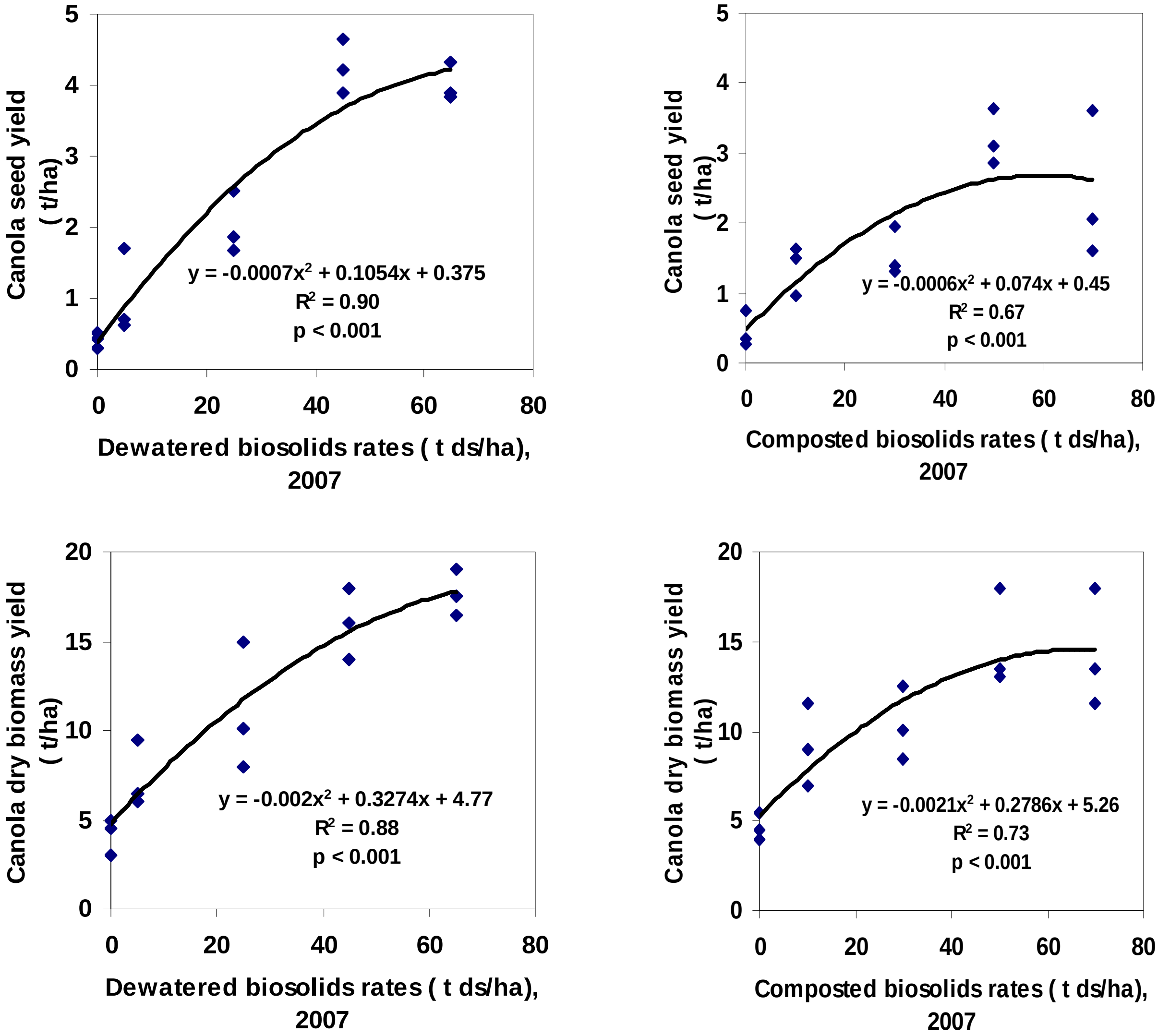


Figure 4.5 The effect of dewatered biosolids and composted biosolids applications on canola seed and dry biomass yields in the 2007 plot experiment. Triplicate measurements for canola seed and dry biomass yields were plotted against biosolids application rates. The probability values stands for the ANOVA (F-test) for the regression line.

Relationship between canola agronomic variables

For canola plots treated with dewatered biosolids in 2006 and 2007, dry seed weight and dry biomass yields were positively correlated (r = 0.88**, r = 0.92**). A positive correlation (r = 0.75**, r = 0.77**) between the number of pods and branches per plant of canola crop was also observed due to dewatered biosolids applications rates in both years of the experiment respectively (Fig. 4.6).

Likewise, the correlations between seed weight and biomass (r = 0.93** and r = 0.85**,) between the number of pods and branches per plant (r = 0.73** and r = 0.89**) in composted biosolids treated canola plots in 2006 and 2007 were also significant and similar to the correlations observed in dewatered biosolids treated canola plots (Fig. 4.7).

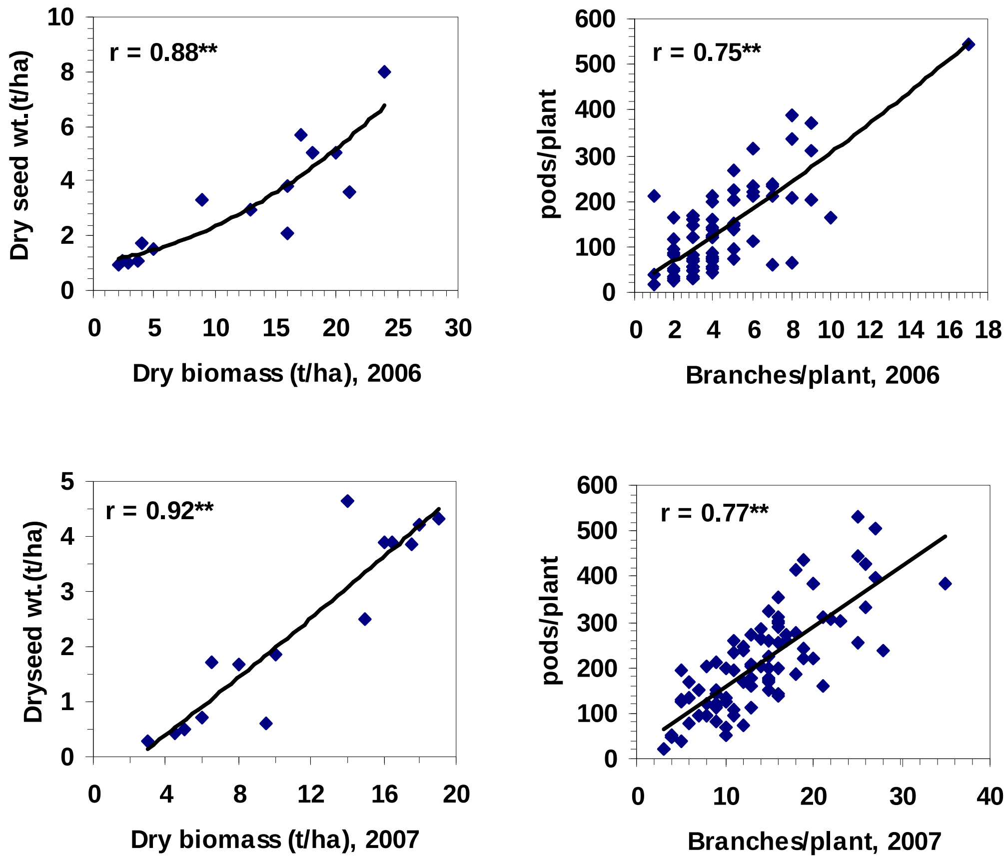


Figure 4.6 Relationships between canola yield components as affected by dewatered biosolids applications rates in 2006 and 2007 field experiment.

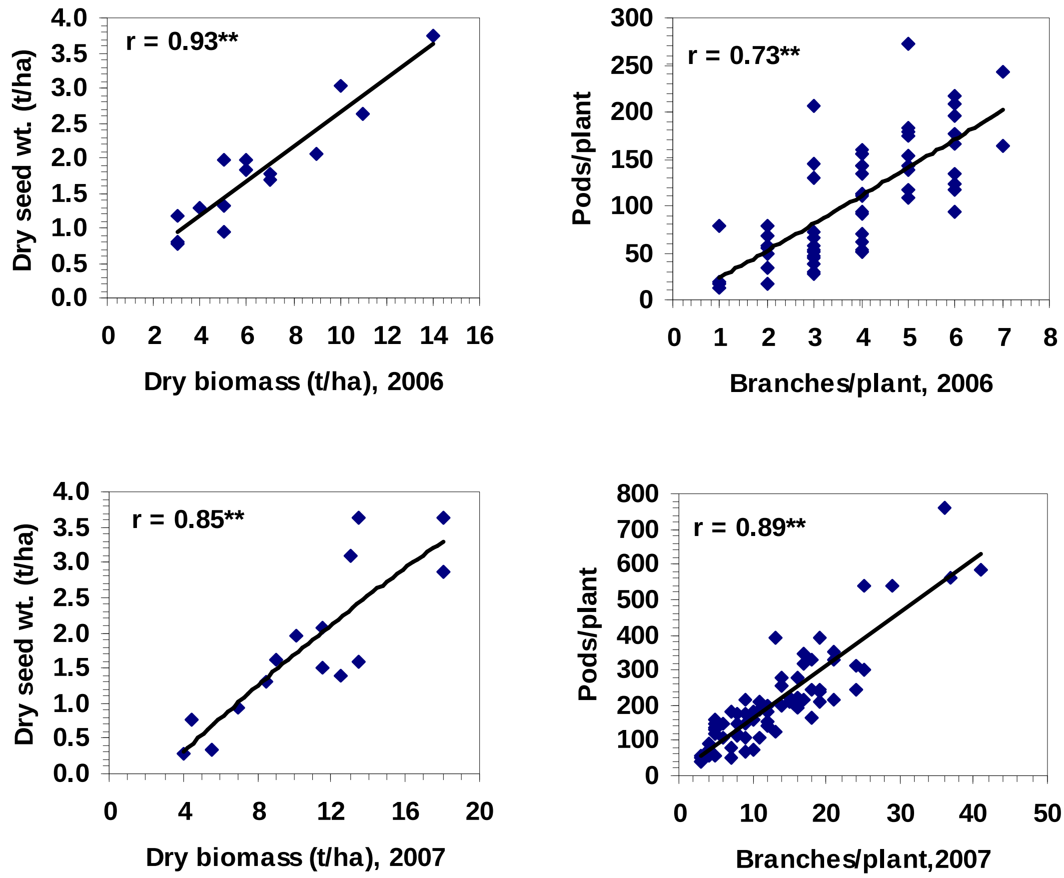


Figure 4.7 Relationships between canola yield components as affected by composted biosolids applications rates in 2006 and 2007 field experiments.

Discussion

In the 2006 experiment, canola seed and plant biomass yields in dewatered biosolids treatments were significantly higher than yields in composted biosolids treatments (Table 4.2). This may be explained because the dewatered biosolids have higher potentially mineralizable total N and S and was the highest in Cu and Zn levels than composted biosolids. These nutrients could have significantly contributed to the yield differences between the two biosolids types.

In 2006, seed and biomass yields of canola increased in response to both types of biosolids products showing a quadratic trend with coefficient of determination values $R^2 = 0.57$ and $R^2 = 0.86$ for dewatered biosolids and $R^2 = 0.58$ and $R^2 = 0.51$ for composted biosolids treated canola plots, respectively (Fig. 4.3), whereas in 2007 seed and biomass response of canola crop showed a quadratic trend with coefficient of determination values $R^2 = 0.90$ and $R^2 = 0.88$ for dewatered biosolids and R^2 =67 and $R^2 = 0.73$ for composted biosolids treated canola plots, respectively (Fig 4.5). There appears to have been a shift in the optimum canola seed yield response to dewatered biosolids applications changing from 25 t/ha in 2006 to 45 t/ha in 2007.

Likewise, the optimum canola seed yield response to composted biosolids applications also changed from 30 t/ha in 2006 to 50 t/ha in 2007. This was not expected since there were significant quantities of total N residue left from the 2006 application. However, such changes might be due to significant loses of plant available NH_4-N and NO_3-N at the end of 2006 through volatilization and dentrification, respectively and total N may not necessarily indicate the plant available N forms.

The other possible explanation might be due to large extent of inter seasonal variations in crop response between the two years which probably mask the response of canola to any residual nutrients left in soil.

In both years of the plot trial the conventional fertilizer treated canola plots produced significantly lower canola seed and biomass yields compared to the 25 t/ha , 45 t/ha and 65 t/ha and 50 t/ha and 70 t/ha dewatered biosolids and composted biosolids treated plots, this was not expected however fertilizers are usually applied to match the crop nutrient requirement, and the significant increase in seed and biomass yields of canola crop obtained from biosolids treated plots would suggest that biosolids application might have more than nutrient requirement, and these may well include organic matter enrichment, supply of sulphur, micronutrients and improve cation exchange capacity and structural stability of the soil.

It might be also since the fertilizers were spread on each of the experimental plots and immediately watered using sprinkler system, it is expected that some of the fertilizer N may have lost through NH_3 volatilization and denitrification processes or through leaching.

There are some variations in seed and biomass yields obtained in the 2006 inorganic fertilizer treated canola plots which was not expected, however such variation were not statistically significant. But, seed and biomass yield of canola recorded in 2006 were slightly different from the yields obtained in 2007, and these variation would be due to seasonal differences between 2006 and 2007 cropping season and environmental parameters such as rainfall, temperature and other growing conditions might have impacted and resulted in some differences in crop yield between 2006 and 2007 plot experiments.

Jackson (2000) fitted very close linear and quadratic relationships between seed yield and available N. The fact that N nutrition is not always equally effective and interacts with environmental conditions has also been reported by other researchers (Andersen *et al.*, 1996).

Butkute *et al.* (2006) reported similar findings in that increased commercial N fertilization rates in the first year effectively increased canola seed yield, but when nitrogen was applied at higher rates in the second year, the seed yield either did not increase or the increase was not significant and the relationship between seed yield and N loading rates was best expressed by a non-linear regression.

The highest application rates of dewatered biosolids and composted biosolids treatments significantly affected the vegetative growth of the canola crop, which in turn influenced the number of pods per plant. Since the number of seeds per pod and 1000 seed weight were relatively similar at various dewatered and composted biosolids application rates, the increase in the number of pods per plant was the main factor in canola seed yield increments. The number of pods per plant is usually a principal factor determining canola seed yields (Ogunlela *et al.*, 1990). Similar studies (Allen and Morgan 1972; Cheema *et al*. 2001; Ozer, 2002) concluded that the number of pods increased with an increase in applied nitrogen fertilizer due to an increase in nitrogen uptake by plants which significantly contributed to pod set. Other studies which also reported similar findings using canola and elevated commercial nitrogen application rates were Scott *et al.*(1973), Singh and Yusuf (1979), Hocking (1987), Wright *et al.*(1988), Hocking and Randall (1997), Bilsborrow *et al*. (1993).

Both types of biosolids treatments also increased the number of branches per plant and height of canola crops, which significantly contributed to higher plant biomass. This is similar to the findings of Allen and Morgan (1972) who reported that increasing rates of N fertilizer increased the number of branches of canola plants.

As expected, when N was over supplied, canola crops at the 65 t/ha dewatered biosolids treated plots the crop experienced lodging which would have contributed to the insignificant seed yield increments in the 2007 experiment, similar to this findings Scott *et al*. (1973) Sheppard and Bates (1980), Bailey (1990) reported that canola frequently encounters lodging, and occurs primarily with taller varieties and under conditions of increasing N rates. This is evidently an indirect effect since high N rates promote the formation of more pods and seeds but also decrease of culm stability impacting pod filling.

4.3. Responses of oats to the applications of biosolids

Figure 4.8 and 4.9 shows oats grown at different growth stages during the 2006 and 2007 experiments conducted at WWSP.

In the 2006 experiment, dewatered and composted biosolids applications significantly increased the seed and plant biomass and yield components of the oat crop (Table 4.4). Relative to the unamended and conventionally fertilized control plots. The yield and yield components of the oat crop significantly increased following application of dewatered biosolids and composted biosolids. However, the 2007 yield records were lower than the yields obtained in the 2006 growing season. This was because in the 2007 experiment, the oat crops were infested with stem rust which impacted the yield and yield components of the crop (Fig. 4.12 and 4.13).

Figure 4.9 Oats grown using dewatered and composted biosolids at Western Water Surbiton Park, 2006 and 2007 field experiments.

Year 1 (2006)

Oat seed and plant biomass yields were significantly affected by dewatered biosolids application rates. The highest oat seed (6.3 t/ha) and dry biomass yields (22.4 t/ha) were recorded at the 45 and 65 t/ha dewatered biosolids application rates were significantly ($p < 0.05$) higher than the yields obtained from unamended control plots. The grain yields (4.9 t/ha) in conventionally fertilized plots were similar with the corresponding seed yield obtained (4.8 t/ha) at the 65 t/ha dewatered biosolids treated plots (Table 4.4). The grain yields in the 25 t/ha treated plots (5.2 t/ha) was also higher than the yield obtained in the 65 t/ha dewatered biosolids treated plots. There was no significant difference in terms oats seed yield between the 25 t/ ha, 45 t/ha and 65 t/ha loading rates, hence the 25 t ds/ha dewatered biosolids application would be the optimum loading rate, as shown in Figure. 11 where the raw data for oat seed and biomass yields were plotted against biosolids applications.

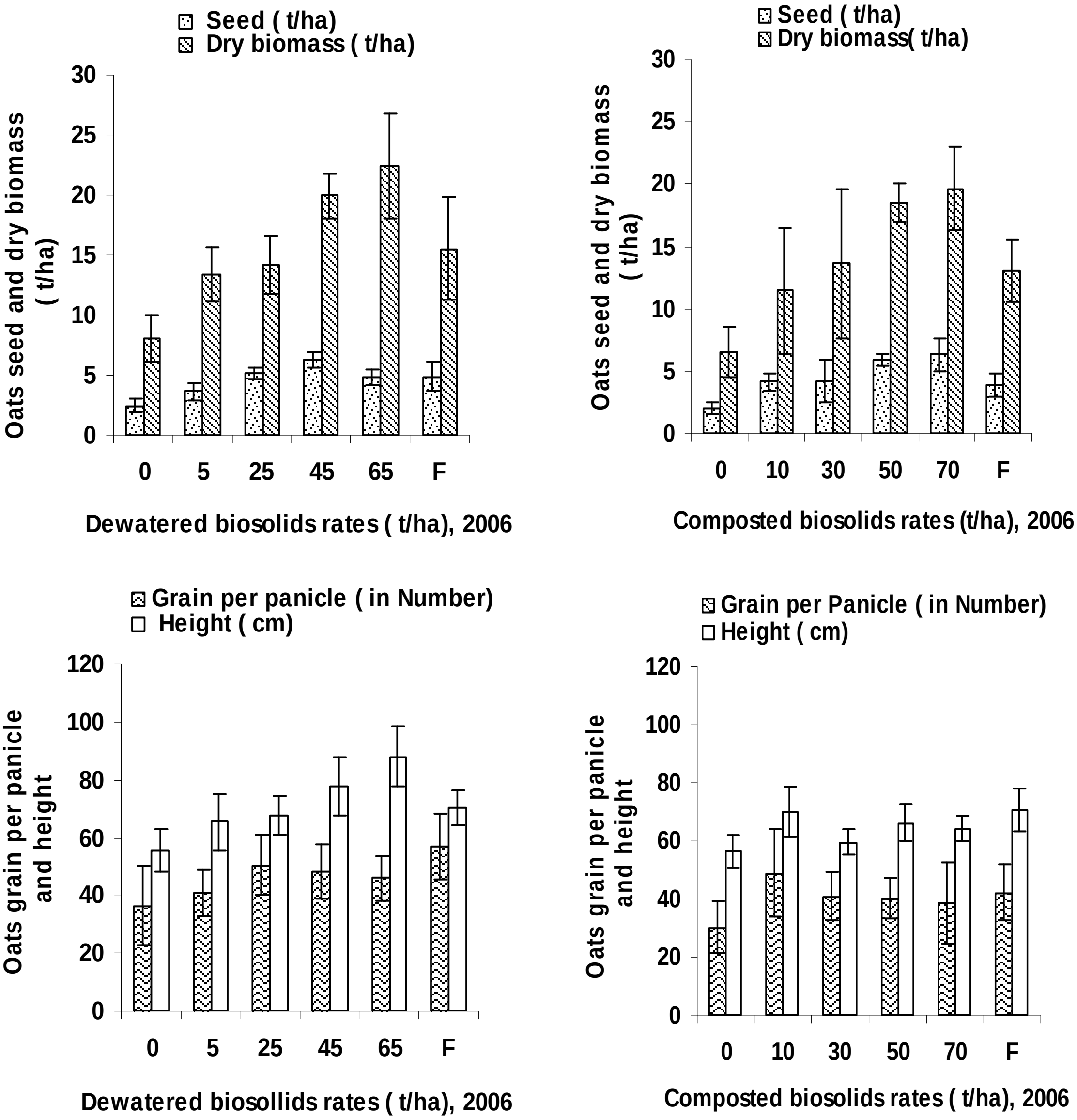


Figure 4.10 Oats seed weight and dry biomass yields and yield components as affected by dewatered and composted biosolids application rates on dry solids basis, (2006). The error bars indicate ± sd of triplicate measurements

Table 4.4 The response of oats to the application of increasing rates of dewatered biosolids and composted biosolids (t/ha) in the 2006 field experiment.

Experiment type	Application rates (t/ha)	Dry seed wt. (t/ha)	Dry biomass (t/ha)	Grain per/panicle[n]	Height (cm)
Dewatered biosolids treated oats plots	Control	2.4	8.	36	55
	Fertilized	4.9	15.5	57	70
	5	3.6	13.4	41	65
	25	5.2[e]	14.2	50	68
	45	6.3	19.9	48	77
	65	4.8	22.4	46	89
	$LSD_{0.05}$	1.6**	6.5*	5.2***	4.5***
Composted biosolids treated oats plots	Control	2.0	6.6	30	56
	Fertilized	3.9	13.1	49	70
	10	4.2	11.4	41	60
	30	4.2	13.6	40	66
	50	5.9[f]	18.5	39	64
	70	6.4	19.6	42	71
	$LSD_{0.05}$	1.98*	8.2*	4.7***	3.1***

The superscripts *, ***, and ns refers to significant treatment effects in ANOVA at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively, whereas the superscript "n" refers to grain per panicle was expressed in number. The superscript 'e' and 'f' refer to the optimum oat seed yield

Compared to the unamended control plots, the number of grain per panicle and plant height increases significantly ($p < 0.001$) as dewatered biosolids application increased. The highest grain per panicle (57) was recorded in the fertilizer treated control plots. Grain per panicle (50) and plant height (89 cm) were recorded at the 25 and 65 t/ha dewatered biosolids treated plots, respectively (Table 4.4).

In composted biosolids treated plots, the highest oat seed (6.4 t/ha) and biomass yields (19.6 t/ha) were recorded at 70 t/ha loading rates and were significantly different from the unamended control plots ($p < 0.05$) (Table 4.4). The highest number of grain per panicle (49) was recorded in the fertilized control plot but the maximum plant height (71 cm) ($p < 0.001$) was attained at the 70 t/ha composted biosolids treated plot (Table 4.4).

Seed and biomass yields of oats recorded at 70 t/ha composted biosolids treated plot were significantly higher than the yields recorded from conventionally fertilized and unamended control plots (Table 4.4). In the 2006 trial, in general oat seed yield results showed that the 50 t/ha ds composted biosolids application would be the optimum loading rate (Fig. 4.11).

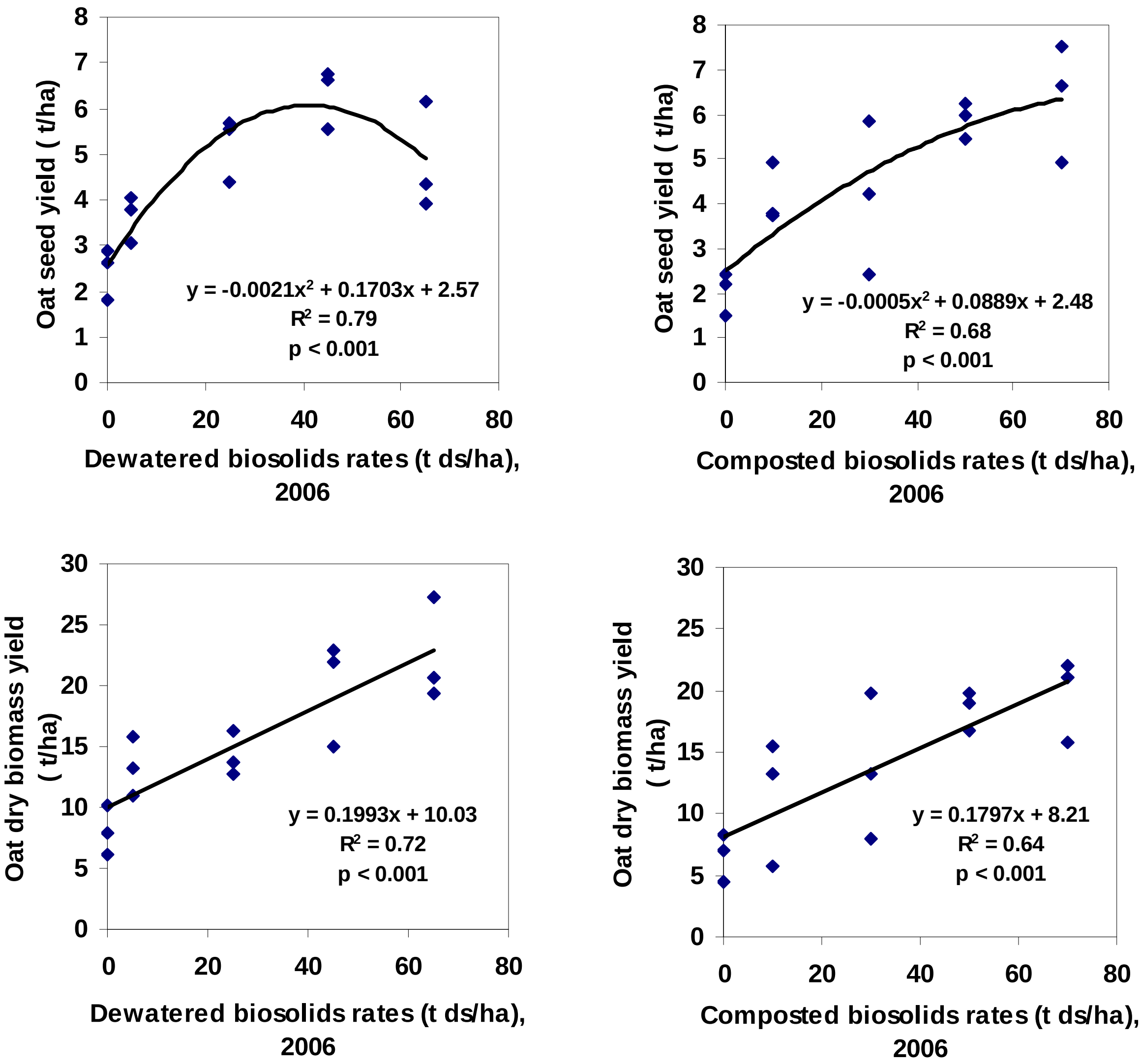


Figure 4.11 The effect of dewatered biosolids and composted biosolids applications on oat seed and dry biomass yields in the 2006 plot trial. Triplicate measurements for canola seed and dry biomass yields were plotted against biosolids application rates. The probability vaues stands for the ANOVA (F-test) for the regression line.

Year 2 (2007)

In 2007 dewatered biosolids treated oats plots, the oat seed yield was different between the treatments. The highest application rate gave about double the seed yield of either the unamended or conventionally fertilized control plots, although not statistically different from the control plots. The oat crops were infested by stem and leaf rust, which reduced the photosynthetic capacity of the crop, thereby negatively impacting the seed setting process. This may have contributed to the decrease in the seed and biomass yields of the crop.

The highest oats plant biomass (15 t/ha) was recorded at the 65 t/ha dewatered biosolids treated oat plots and this was higher ($p < 0.05$) from the control plot. Grain per panicle and plant height of the oats crop recorded at the 45 t/ha dewatered biosolids application rates were significantly ($p < 0.001$) higher than the control plot (Table 4.5).

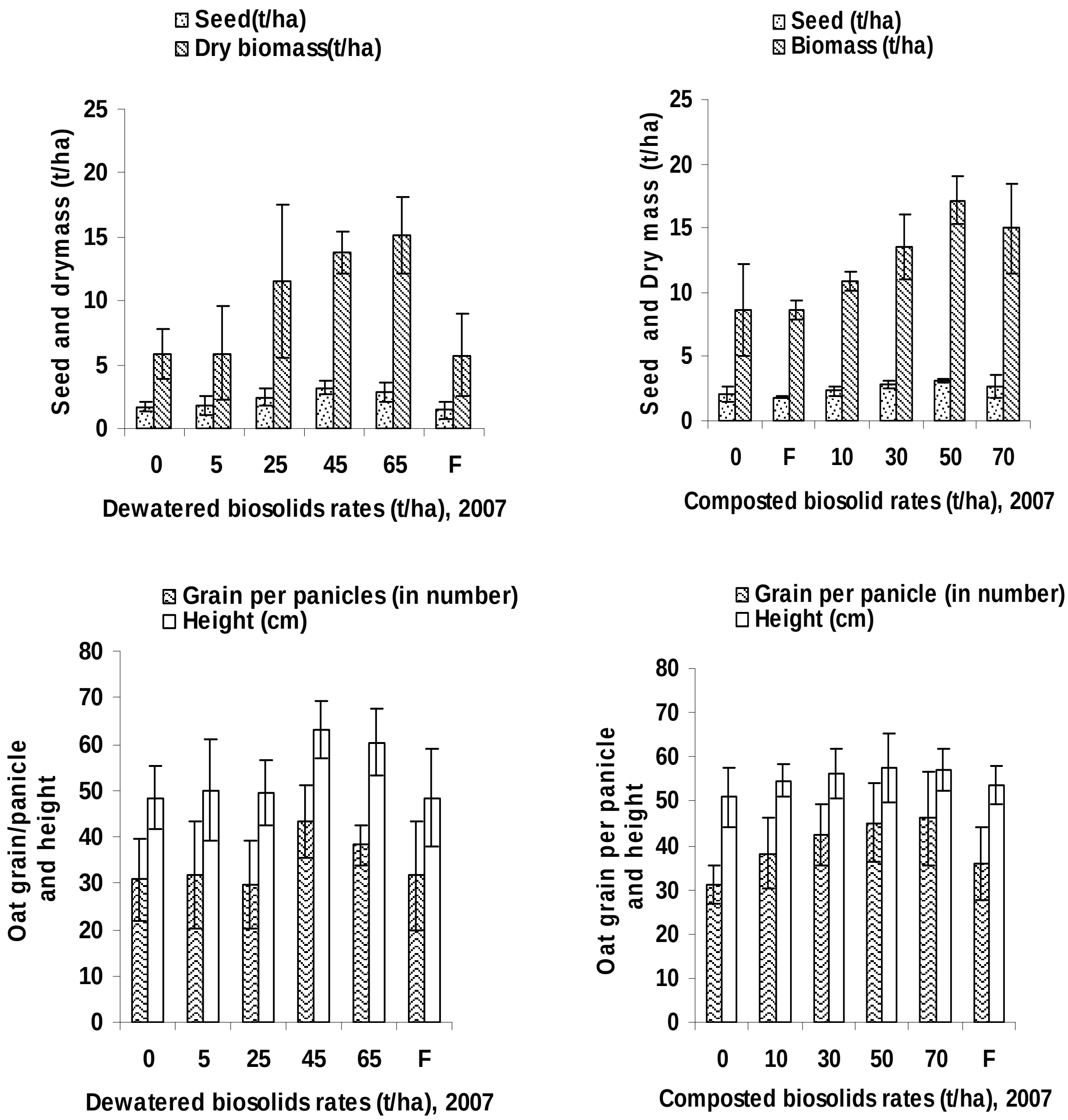


Figure 4.12 Oats seed and dry biomass yields and yield components as affected by dewatered and composted biosolids application rates on dry solids basis, (2007).

Table 4.5 The response of oat to the application of various rates of dewatered and composted biosolids (t/ha) in the 2007 field experiment.

Experiment type	Application rates (t/ha)	Dry seed wt. (t/ha)	Dry biomass (t/ha)	Grain per/ panicle$_n$	Height (cm)
Dewatered biosolids treated oats plots	Control	1.7	5.8	31	48
	Fertilized	1.5	5.8	32	48
	5	1.8	5.9	32	50
	25	2.4	12.0	30	50
	45	3.2	14.0	43	63
	65	2.9	15.1	38	60
	$LSD_{0.05}$	ns	7.4*	5.6***	6.1***
Composted biosolids treated oats plots	Control	2.1	8.6	31	51
	Fertilized	1.8	8.7	36	54
	10	2.3	11	38	55
	30	2.9	14	42	56
	50	3.1	17	45	58
	70	2.7	15	46	57
	$LSD_{0.05}$	ns	5.5*	5.73***	4.1*

The superscripts *, *** and ns refers to significant treatment effects in ANOVA at $p < 0.05$, $p < 0.001$, and not significant, respectively, whereas the subscript 'n' refers to grain per panicle was expressed in number.

In the composted biosolids trial, oats seed yield was not significantly different from the control plot. However, the highest ($p < 0.05$) oats plant biomass 17 t/ha was recorded at the 50 t/ha composted biosolids rates (Table 4.5). Figure 4.13 shows the raw data for oat seed and biomass yields plotted against biosolids applications.

Grain per panicle and plant height recorded at the 70 t/ha composted biosolids treated plot were significantly ($p < 0.05$) different from the control plot.

The fertilized and unfertilized control plots gave relatively similar results in terms of crop biomass, grain per panicle and plant height measurements in the 2007 composted biosolids treated oat plot.

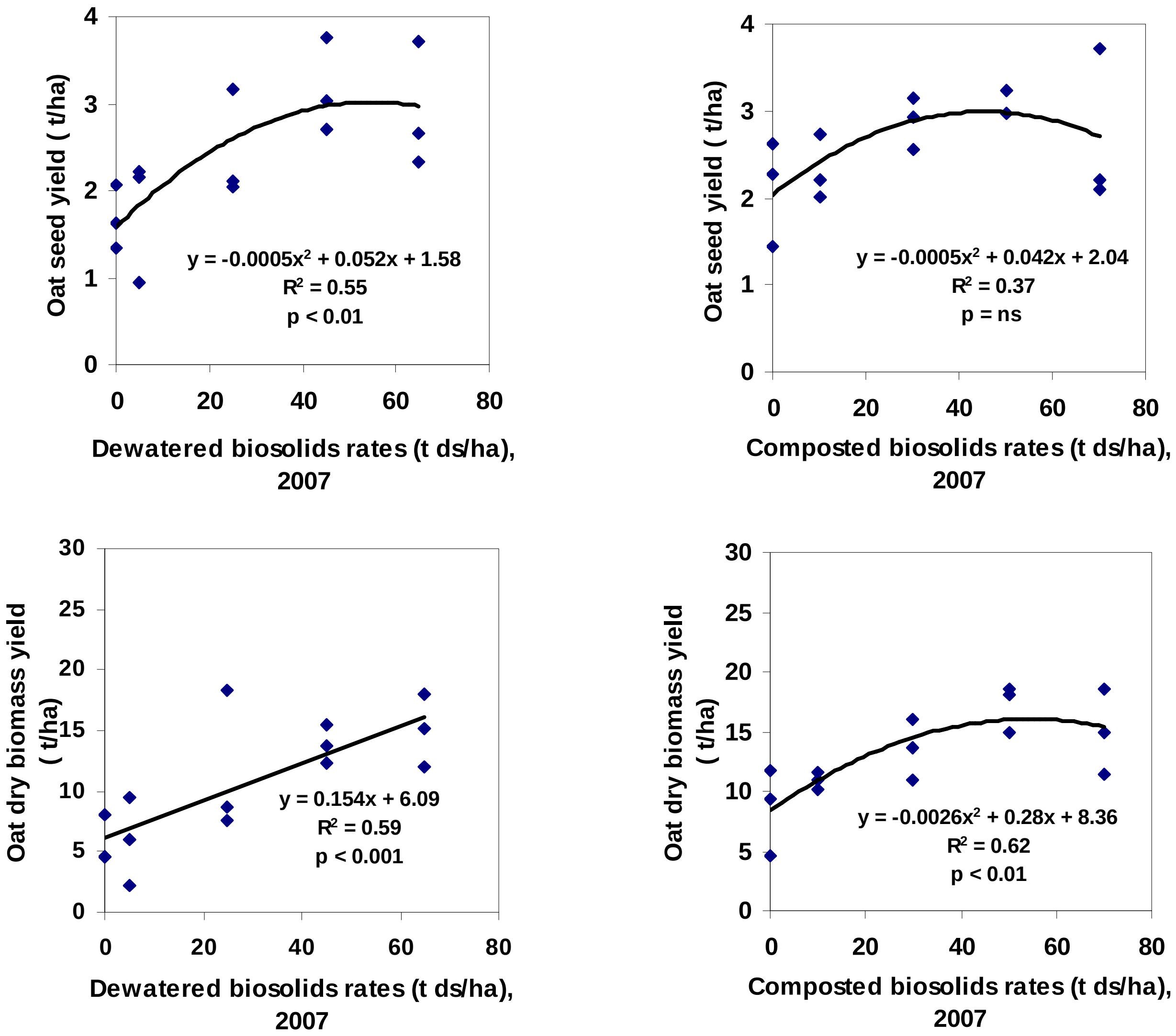


Figure 4.13 The effect of dewatered biosolids and composted biosolids applications on oat seed and dry biomass yields in the 2007 plot trial. Triplicate measurements for oat seed and dry biomass yields were plotted against biosolids application rates. The probability vaues stands for the ANOVA (F-test) for the regression line.

Relationships among oats agronomic variables

Figures 4.14 and 4.15 show the relationship between the various agronomic variables for oats grown using dewatered and composted biosolids in 2006 and 2007 experiments.

Although, the correlations between the number of grain per panicle and height of oats in the 2006 dewatered biosolids treated oats plots was poor ($r = 0.24$), the correlations between seed and plant biomass of oats was significant ($r = 0.67^{**}$). Similar results were also observed in 2007 where seed and plant biomass of oats crops treated with dewatered biosolids were poorly correlated (r = 0.47), however, the number of grain per panicle and plant height was positively correlated (r = 0.70**) (Fig. 4.14).

For the 2006 and 2007 composted biosolds treated oats plots, oats seed and biomass was positively correlated (r = 0.77** and r = 0.68**) respectively, however, grain per panicle and plant height were poorly correlated (r = 0.26 and r = 0.42) for the two years respectively (Fig.4.15).

The positive correlations between yield and yield components of canola and oats crop indicated that the crops responded to dewatered and composted biosolids plant nutrients (such as N, P, S, Cu, and Zn) contained in the biosolids. These positive correlations between crop yield and yield components suggests the contribution of each yield components to seed and biomass yield increases quadratically due to dewatered biosolids and composted biosolids loading rates.

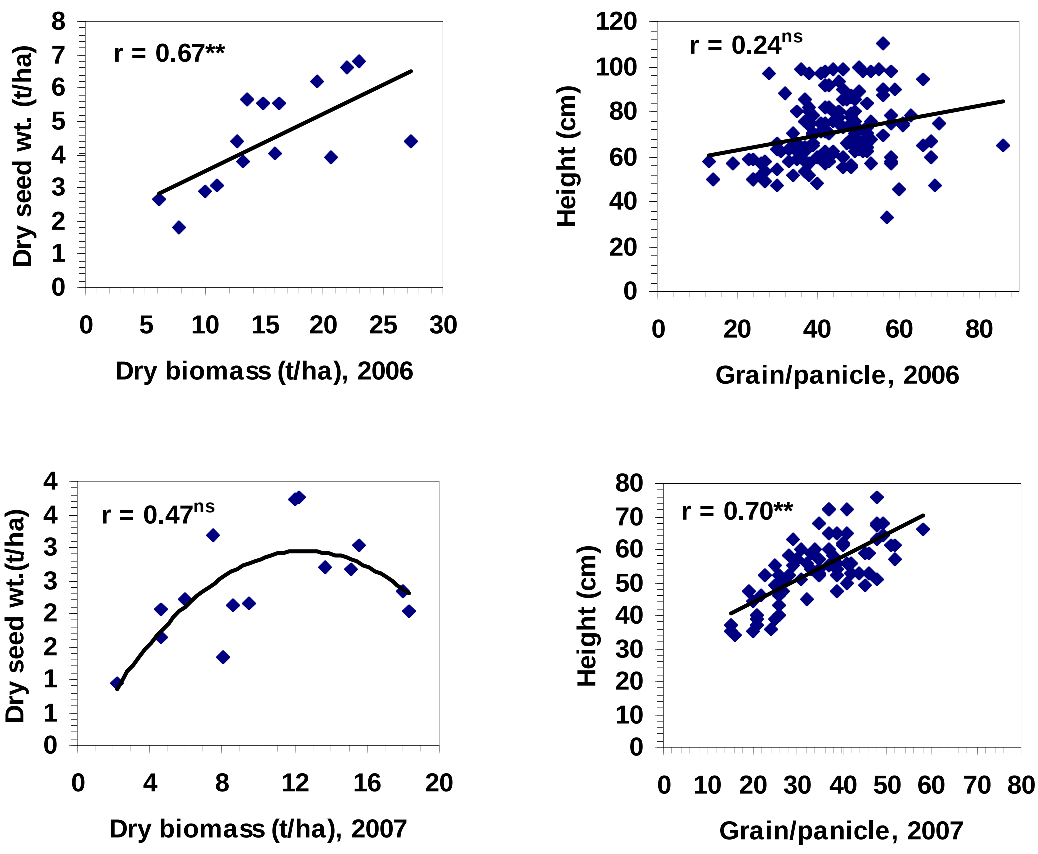


Figure 4.14 Correlations between yield and yield components of oats as affected by dewatered biosolids applications rates in 2006 and 2007 field experiments.

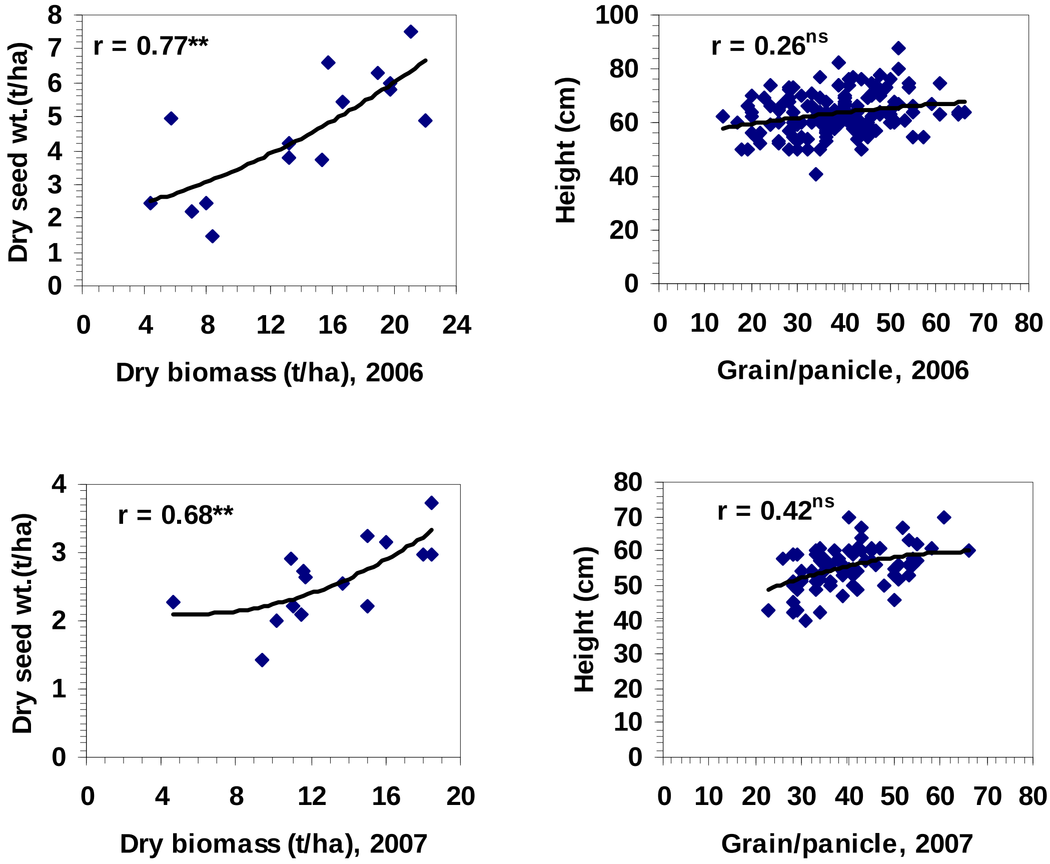


Figure 4.15 Correlations between yield and yield components of oats as affected by composted biosolids applications rates in 2006 and 2007 field experiments

Discussion

At biosolids rates increased, the oats crops were tall with increased number of grains per panicle which had a significant effect on seed and biomass yields. Nevertheless, dewatered biosolids and composted biosolids rates had relatively a similar effect on seed and biomass yields, grain per panicle and plant height (Table 4.4 and 4.5). The concentration of soil background P level was adequate, thus the observed positive response of oats to the application of both biosolids types was expected to be due to the interaction effects of high concentrations of N, S, Cu and Zn contained in the biosolids and the sufficient water available to the crops through sprinkler watering system.

Several researchers suggested various fertilizer rates for optimum oats seed production (Hamill, 2002 ; Entz *et al.*, 2004; Weightman *et al.*, 2004) particularly for yield optimization and lodging control. A number of researchers also showed that nitrogen management is crucial to oats production (Brinkman and Rho, 1984; Marshall *et al.*, 1987). Nitrogen directly influences yield by affecting the various yield components, such as panicle density and kernel number, as well as dry matter production and harvest index. The relative contribution of each yield component in response to increased nitrogen level will change depending on the levels of N used and environmental conditions (Brinkman and Rho, 1984; Marshall *et al*., 1987; Anderson and McLean, 1989; Hamill, 2002).

In the 2006 plot trial, oats seed and biomass yield showed a quadratic trend with coefficient of determination values R^2 = 0. 79 and R^2 =72 in response to dewatered biosolids applications, respectively, whereas, in the 2006 composted biosolids treated oats plots, oat seed and biomass yield increased showing a quadratic trend with coefficient of determination values R^2 = 68 and

R^2 = 64 respectively (Fig. 4.11). Similarly, in 2007, oat seed and biomass yields slightly increased, both showed a quadratic trend R^2 = 0.55 and R^2 = 0.59 following dewatered biosolids loading rates. But in the 2007 composted biosolids treated plots the R^2 = 37 was not significant, however, oat biomass yields showed significant increase and showed a quadratic trend with

R^2 = 62 (Fig. 4.13).

In 2007, oats seed and biomass yield recorded from dewatered biosolids and composted biosolids treated oats plots were relatively similar and not significantly different from the unamended control plot (Table 4.5).

4.4. Effect of crop rotation

Although the inclusion of canola with cereal crops under rotation usually offers yield benefits (Angus et al., 1999; Kirkegaard et al., 2000), in this study, due to a stem rust infestation of the 2007 oats plots, it was difficult to quantify the seed yield benefits of canola for the subsequent oats crop.

4.5. Comparisons of crop yield responses between biosolids types

In the 2007 experiment after repeated application of both biosolids types, slight increases in seed and plant biomass yields of canola were observed, however these increments when compared with the 2006 recordings were not significantly different ($p < 0.05$).

Dewatered biosolids application rates of 25, 45, 65 t/ha (Table 3.2) and composted biosolids application rates of 30, 50 and 70 t/ha (Table 3.3) gave significantly higher canola seed and biomass yields in both years than conventionally fertilized control plots.

Unlike the 2006 results, the number of seeds/pod in the 2007 dewatered and composted biosolids treated canola plots was significantly higher than the control plots suggesting a significant impact of biosolids on the number of seeds per pod.

As expected, yield responses of canola to the applications of dewatered biosolids were significantly higher than the yields obtained from composted biosolids treated plots, since dewatered biosolids had higher plant available nutrients than composted biosolids (Fig. 4.2, 4.3 and 4.4).

During the first year of the experiment, oats seed yield obtained at 45 t/ha dewatered biosolids treated plot was not different from the corresponding oats seed yield recorded at 70 t/ha composted biosolids application rates. Despite the highest number of grain per panicle (57) being recorded from the conventionally fertilized control plots the oats seed yield recorded at the 65 t/ha dewatered biosolids rates was not different from the seed yields obtained from conventionally fertilized control plots.

Both types of biosolids had a considerable positive impact on oats seed yields (Fig. 4.10. 4.11, 4.12 and 4.13), however, a comparison between the two years of the experiments were not possible because of the incident of stem rust infestations that the oats crop experienced during the second year of the experiment.

In general, dewatered biosolids rates (45 and 65 t/ha) and composted biosolids rates (50 and 70 t/ha) gave significantly higher seed and biomass yields than conventionally fertilized canola and oats plots in both years of the experiments, this could possibly be due to other factors such as the interaction between sprinkler irrigation with high biosolids nutrients may have significantly influenced the yield responses better than the results obtained from conventionally fertilized plots.

4.6. Effect of biosolids application on soil temperature and seed germination

Canola crop

After 2-3 weeks of crop establishment during the 2007 biosolids trial, soil temperature data were recorded from the 72 biosolids treated and control plots of canola and oat crops; 10 temperature readings per plot at 10 cm soil depth were taken. Temperature readings for canola were taken early in the morning at 7 am, whereas for oats it was taken at 1.30 pm in the after noon. Figure 4.16 depicts seedlings stages of canola and oats crops after 4-5 weeks of crop establishment.

The numbers of canola and oats seedlings were also counted from a sample of a 1 m^2 quadrant from the centre of the plot. Figure 4.16 presents seedling emergencies of canola and oats crops at 4-5 weeks after sowing in the 2007 field experiments at (WWSP).

When the number of seedlings recorded in the control and the highest biosolids receiving plots were compared, there was a significant ($p < 0.05$) difference in the percentage of canola seedlings due to dewatered and composted biosolids application. Dewatered biosolids and composted biosolids loading rates had a significant ($p < 0.001$) effect in changing the soil temperature from 6.2 control plot to 8.3 ^{0}C (65 t/ha plot) for dewatered and from 5.6 control plot to 8.2 ^{0}C (70 t/ha plot) for composted biosolids treated canola plots (Fig. 4. 17).

Several researchers reported different temperature ranges for canola seed to germinate and emphasized the significance of temperature on canola seedlings germination; for instance Thomas (1984) reported the optimum germination temperature for canola was 10-30^{0}C and Kondra *et al*. (1983) suggested 15-20 ^{0}C.

In this study, incorporation of dewatered and composted biosolids into the soil significantly increased the soil temperature which had a positive impact on the germination of canola seedlings.

The fact that cold temperatures have negative impact on canola seedlings germination was also reported by several researchers in which case spring canola seeded into sub optimal soil temperatures had lower emergence and stand establishment rates due to the seed rotting in the cold soils (Blackshaw, 1991; Kondra *et al*., 1983; Livingston and deJong, 1990).

The importance of temperature for germination of canola seedlings was also emphasized by Morrison *et al*. (1989) where 5 ^{0}C was reported as the base temperature (below which little significant plant growth occurs) in a controlled environment.

The number of canola seedling germinated per metre square in the control plots were 37 % whereas for the highest dewatered and composted biosolids treated canola plots were 91 and 83 % respectively. There was also a positive correlation between the number of canola seedlings/m^2 and dewatered and composted biosolids application rates ($r = 0.77^*$, $r = 0.72^{**}$,) for dewatered and composted biosolids treated canola plots respectively (Fig. 4.17).

Qasim *et al*. (2001) investigated the effect of sewage sludge (0, 10 20, 30 40 and 50 t/ha dry wt.), NPK (120:90:30 kg/ha) application rates on the growth of maize crop and found that the lowest germination percentage was found in the control and the highest application rates (50 t/ha) sludge amendments.

Joanna and Wester (2004) conducted a two years green house and field experiments to investigate the effect of applying 34 t/ha biosolids on soil water, soil temperature, and seedling emergence and growth of blue grama (Bouteloua gracilis) and green sprangletop (Leptochloa dubia) in a Chihuahuan desert grassland. Biosolids usually increased minimum and reduced maximum temperatures. Biosolids also generally reduced soil water loss. They concluded that when environmental conditions are neither extremely unfavorable nor extremely favorable, the presence of surface-applied biosolids and the resulting reduction in soil water evaporation and moderation of soil temperature extremes may provide conditions conducive for successful emergence.

Giuseppe and Giovanni (1993) investigated the effect of digested sewage sludge application at a rate of 0.5, 1 and 2 kg/m^2 and commercial fertilizer on the germination and yield of peas and broad bean under field condition. They reported that relative to the control plots the presence of sludges in the soil improved germination and yield of both species to the same extent as did the fertilizer. The use of sludge improved the total production of both species above that of both the control and the fertilizer tests.

Murillo *et al*. (1995) evaluated the effect of two urban composts on germination of ryegrass and sunflower and seedling performances in pot experiment. From their findings concluded that compared with the control, coarse matured urban compost treatments (100 %) increased the germination index of the crops as a result of enhanced root development.

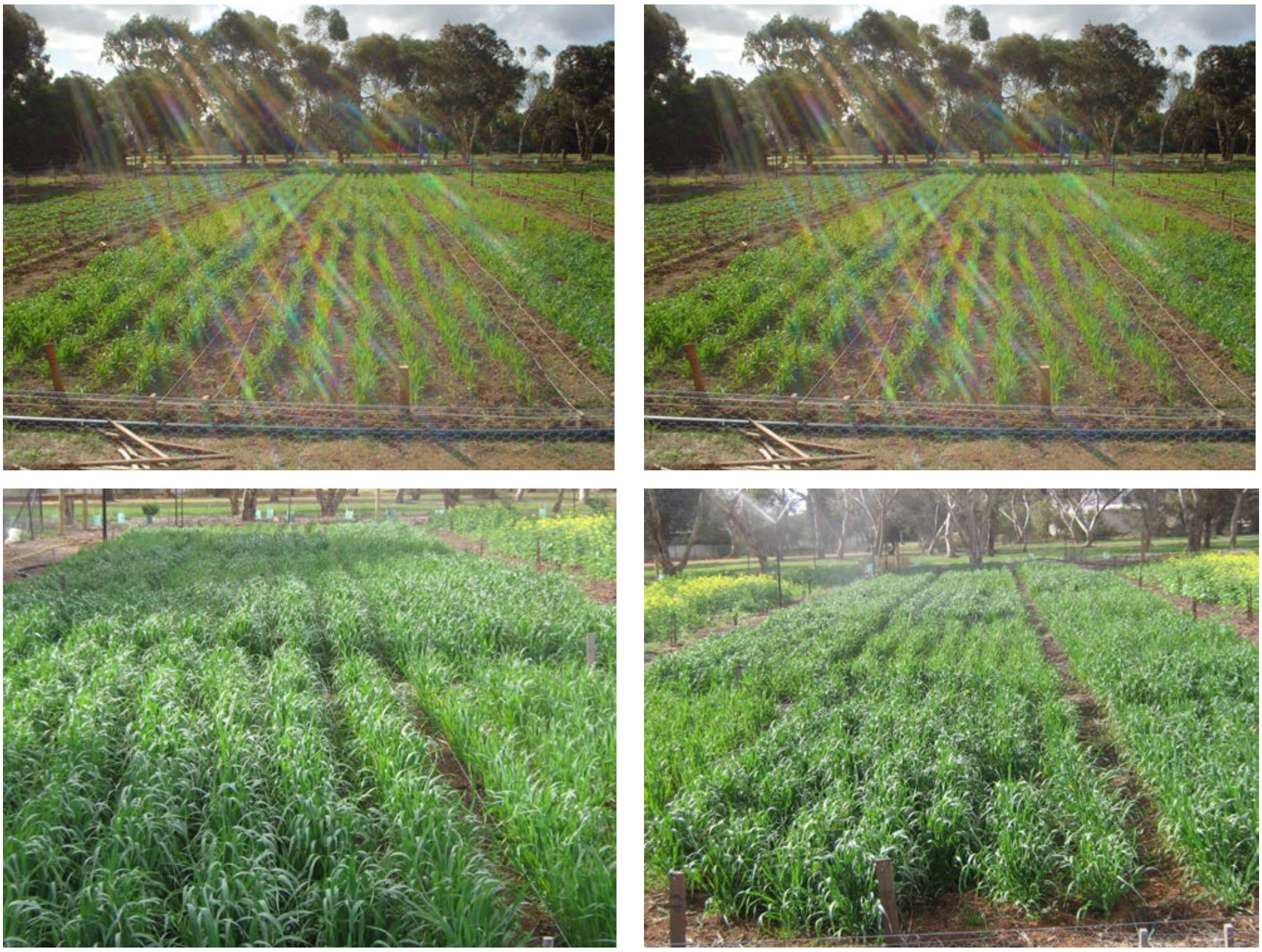


Figure 4.16 Canola and oats crops seedlings at 4-5 weeks after sowing in the 2007 field experiments at Western Water Surbiton Park (WWSP)

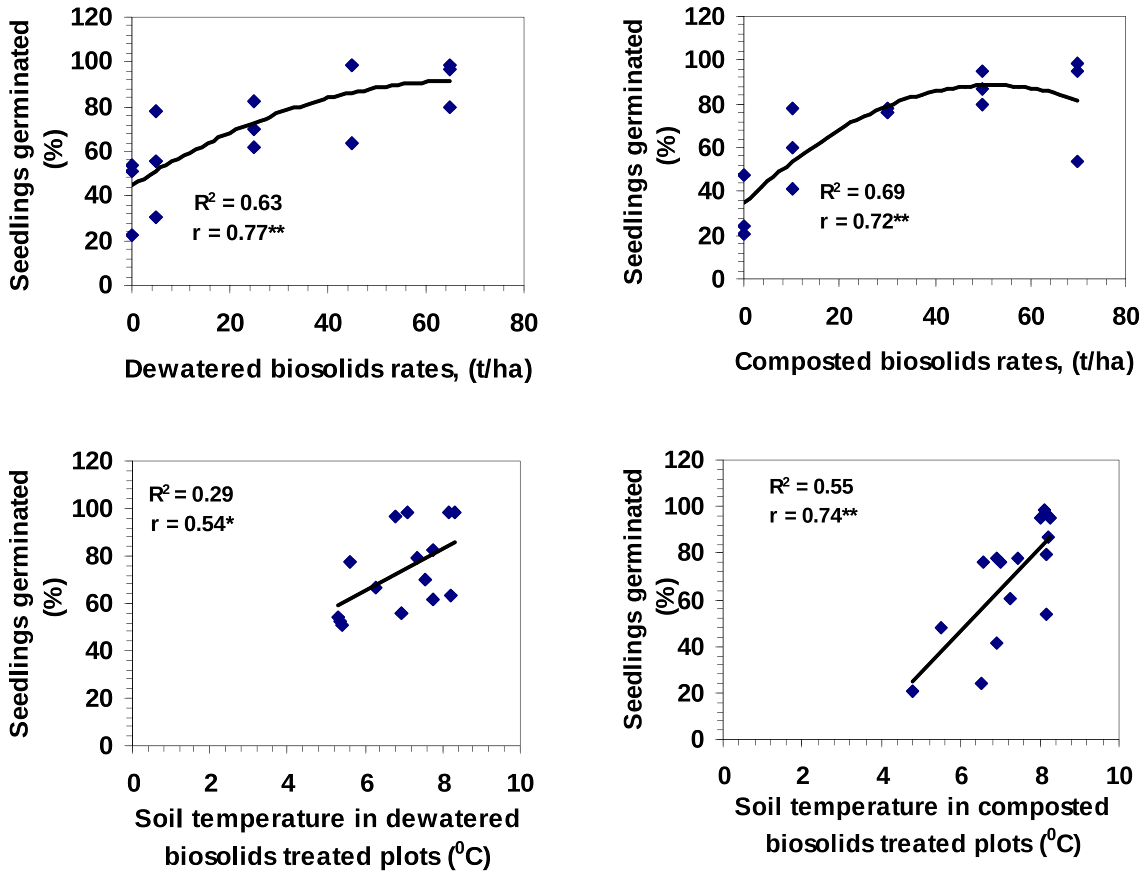


Figure 4.17 Effect of dewatered biosolids and composted biosolids application rates on soil temperature and canola seed germination, in the 2007 field experiment.

Given the research in the literature, it is likely that, in this study, dewatered biosolids and composted biosolids application rates changed the heat capacity of the soil as evidenced by the significant changes in soil temperature observed, creating favourable conditions for canola seeds to geminate effectively (Appendix E Table E-1).

Oat crop

For oats treated with dewatered biosolids and composted biosolids plots, biosolids loadings had no significant effect on seed germinations. Germination of oats can occur at 3-5 ^{0}C, an indication they can tolerate early season cold temperatures. Oats perform best in a cool and moist climate since they require more moisture to yield a given unit of dry matter than any other cereal except rice (Welch, 1995).

The percentages of oats seedlings germinated were uniform as biosolids loading rates increased. However, significant differences ($p < 0.01$) between the percent seedlings germinated in dewatered biosolids and composted biosolids treated oat plots were observed, with the dewatered biosolids treated plots having a slightly higher germination percentage than composted biosolids treated plots (data not shown).

4.7. The impact of biosolids application on N, P, S and oil concentrations in canola seed.

Canola seed samples taken from the various dewatered biosolids and composted biosolids treated plots in 2006 were analysed for N, P, S and oil concentrations (Table 4.6). The results show that N, P and S in canola seed increased following both types of biosolids applications. When the control plots and the highest dewatered biosolids receiving plots were compared, Kjeldahl N increased from 2.9 to 4.16 %, total P increased from 6200 to 9100 µg/g and total S increased from 3400 to 4600 µg/g respectively.

Despite significant increases in the quantity of canola seed observed due to biosolids applications, canola seed oil concentration decreased from 46.5 (control plot) to 37.2 % (65 t/ha plot) following dewatered biosolids applications.

A relatively similar trend was also observed for canola seed obtained from composted biosolids treated plots in which case Kjeldahl N increased from 2.9 to 3.2 %, total P increased from 6200 to 8900 µg/g, total S increased from 3400 to 3800 µg/g respectively, but, seed oil concentration decreased from 46.5 to 43.4 % in response to composted biosolids applications (Table 4.6).

Table 4.6 The impact of biosolids application on N, P, S and oil concentrations in canola seeds in 2006 field experiment

Effect of dewatered biosolids				
Rates (ds t/ha)	TN (%)	P µg/g	S µg/g	Oil (%)
0	2.91	6200	3400	46.5
5	3.08	7800	3400	45.2
25	3.47	8600	4200	42.2
45	3.60	8300	4200	41.9
65	4.16	9100	4600	37.2
Fertilized plots	3.19	5600	3900	43.9
Effect of Composted biosolids				
Rates (ds t/ha)	TN (%)	P µg/g	S µg/g	Oil (%)
0	2.91	6200	3400	46.5
10	2.97	8600	3200	45.4
30	2.81	8200	3200	47.5
50	3.13	8900	3800	44.5
70	3.22	8700	3800	43.4
Fertilized plots	3.19	5600	3900	43.9

Dewatered biosolids had greater effect in changing the levels of N, S and oil concentrations than composted biosolids; this was expected since dewatered biosolids had significantly higher concentrations of total N and total S than composted biosolids at the beginning of the study. Despite higher levels of P in composted biosolids than in dewatered biosolids, higher levels of P in canola seeds treated with dewatered biosolids was observed indicating that P contained in dewatered biosolids was more bioavailable than the P in composted biosolids.

Increases in biosolids application rates increased N in canola seed but decreased seed oil concentration showing an inverse relationship. Likewise, Brennan *et al*. (2000) observed a negative correlation between canola seed oil concentrations and protein content following nitrogen application rates. Similar findings were also reported by Pritchard *et al*. (2000) and Norton (1993) where a strong inverse relationship between canola seed oil concentration and seed protein content were noted under Wimmera conditions in Victoria.

However, the decrease in seed oil concentration was compensated by increases in the quantity of oil yields due to increasing seed yield as application rates were increased.

4.8. Conclusion

Incorporation of biosolids into crop land significantly increased the seed and plant biomass yields of both canola and oats. Although the conventionally fertilizers were applied at recommended rate, it may not be optimal for the specific site conditions. Thus, the increase in yield and yield components of both crops due to biosolids application surpassed that of conventionally fertilized control plots. These increases in the yield components significantly contributed to the total seed and biomass yields increments of both crops. Canola seed yields were optimum at 25 t/ha dewatered and at 30 t/ha composted biosolids loading rates in the 2006 biosolids application rates, whereas, the optimum oats seed yield in the 2006 trial were recorded at the 25 t/ha dewatered and 50 t/ha composted biosolids rates, respectively.

In the 2007 trial, the optimum canola seed yield were recorded at the 45 t/ha dewatered and 50 t/ha composted biosolids rates, respectively. The optimum dewatered and composted biosolids rates in 2007 were shifted (increased) for both biosolids types, and this was not expected, however such increases in the optimum biosolids rates might be due to inter seasonal variations in the response of the two crops to biosolids nutrients.

Though the yields obtained at the higher 65 t/ha and 70 t/ha dewatered biosolids and composted biosolids rates seem slightly higher than the other loading rates, they were not significantly different from the optimum dewatered biosolids and composted biosolids application rates.

The response of canola to the application of biosolids in terms of yield and yield components was greater than oats crops for dewatered biosolids and composted biosolids in both years of the experiments. Since the dewatered biosolids had significantly higher concentrations of total-N, NH_4-N, S, Cu and Zn, as expected the influence of dewatered biosolids on canola grain yield was higher than composted biosolids, although, yield responses of oats to both biosolids types were not significantly different.

The highest canola seed yields recorded in the 65 t/ha dewatered biosolids rate in the 2006 experiment were not significantly different from the highest 2007 canola seed yield observed at the 45 t/ha dewatered biosolids rate.

From the findings of this study, it can be suggested that the two biosolids types had different physical properties and nutrient compositions and behaviours, and thus the relevance and assumption of using NLBAR (nitrogen limited biosolids application rate) will not be valid for all types of biosolids products, moreover nutrient release properties of the two biosolids could be different and possibly depends on other environmental factors including temperature, moisture, crop and soil types. Therefore, it is suggested that biosolids application should take into account the soil and biosolids type and the specific site characteristics of the area receiving the biosolids.

Biosolids application rates had significant effect on the number of canola seedlings germinated, but the response of oats to biosolids loadings in terms of seedlings germination were not significant. In this study, there is clear evidence that the application of biosolids significantly affected the soil temperature and hence canola seed germination; nevertheless, this needs further investigation.

In this study, biosolids applications increased the concentrations of total N, P and S in canola seed oil; however canola seed oil concentration was decreased as biosolids application rates increased and was negatively correlated with levels of total N in canola seed; nevertheless, the significant increase in seed yield due to biosolids nitrogen compensates for any decrease in oil concentration.

Depending on the growing season, seed oil concentration of Australia canola fluctuates from less than 35% to over 45%. On the basis of a bonification scheme, Eastern Australian farmers are paid for their canola crop, which provides a bonus of 1.5% for every percentage point above 40% oil, on as received moisture content. For a crop with 46% oil, the farmer would be paid a 9% bonus for a crop with 46 % oil content (Mailer ***et. al***., 1998).

Although canola seed oil content vary from year to year depending on climatic conditions, the higher rainfall areas in Victoria and southern New South Wales generally produce the highest oil contents. Due to the lower canola seed oil content in northern areas of Western Australia and New South Wales are a problem which prevents the expansion of the crop to the north (Mailer ***et. al***., 1998).

Although the addition of both biosolids type expected to produce slightly higher oil yields, more N loading had negatively impacted the quality of canola seed oil , dewatered biosolids rates at 5 t/ha and composted biosolids rates at 50 t/ha produced the highest canola seed oil content (45.2 and 47.5 %), respectively, and thus these application rates would be ideal for farmers who need to maximize the oil content to benefit from the bonification scheme.

The study has clearly indicated that biosolids applications had significant agronomic benefits. However, protecting the environment and human health from the build up of excess heavy metals in biosolids amended soil and plants is crucially important. This is addressed in the next chapter.

5

CHAPTER 5. BIOAVAILABILITY AND ACCUMULATION OF HEAVY METALS IN PLANTS AND IN BIOSOLIDS AMENDED SOIL

Introduction

Although total heavy metal concentration provides little indication of the specific bioavailabi lity, mobility, and reactivity in biosolids amended soil, total concentration of heavy metals is an essential indicator of soil deficiency and/or contamination. Evaluation of total heavy metal levels may also be used as a measure for global index of contamination (Alva *et al*., 2000; McLaughlin *et al*., 2000; Walter *et al*., 2002).

The DTPA soil test has been developed to assess pollution (as opposed to contamination) of soil by heavy metals (Clayton and Tiller, 1979). The DTPA extraction procedure provides information about metal solubility and tends to correlate with metal uptake by plants usually in conventionally fertilized plots (Bidwell and Dowdy, 1987; Sommers et al., 1991; Hooda and Alloway, 1994). Hence, DTPA-extractable Cu, Zn, Mn, Fe and Ni can be used as an indicator of bioavailability and potential toxicity of these heavy metals (Soltanpour, 1991). Metals extracted by DTPA mainly exist in exchangeable, organically complexed and carbonate forms as discussed in section 2.6.3.

Although Cadmium is a key element in sludge and a major concern in Australia due to the increased uptake of this element from saline soils, the cadmium concentrations in the dewatered biosolids and composted biosolids used in this plot trial was very low and below detection limits which is consistent with Western water's report and hence was not considered in this experimental study.

The combined analysis of the total and bioavailable fractions of heavy metals in a biosolids amended soil would help to understand the behaviour, mobility and accumulations in the soil, hence, this study has given a considerable emphasis to the residuals of Cu, Zn, Mn, Fe, Ni and Pb both in the biosolids amended soil and plants grown on them.

This chapter reports an investigation into the effect of various biosolids application rates on soil pH, EC and heavy metal availability hence plant uptake of heavy metals under a canola and oats crop rotation regime. It also describes the effect of cropping sequence on the levels of heavy metals residues accumulated following dewatered and composted biosolids applications over the two years period of time.

Total heavy metal concentrations in biosolids amended soil were determined using XRF, whereas the bioavailable fractions were extracted by the DTPA procedure as described in section 3.6 and quantified using ICP-MS. Heavy metals in plants were extracted using HNO_3/H_2O_2 as described in section 3.7 and analysed by ICP-MS.

5.1. The effect of biosolids applications on XRF determined total heavy metals

The XRF procedure was chosen for total heavy metals analysis in soil and biosolids samples, because of its simplicity of sample preparation, speed and ease of operation as well as high precision of the instrument. The results presented in Appendix B also showed that XRF gave a much better estimate of total metals than ICPMS for these types of samples (Appendix B Section B.5).

To examine the impacts of different biosolids types and application rates on the levels of total heavy metal residues accumulated, 30 soil cores per plot at 0-10 cm depth were taken to make one composite sample and hence a total of 72 samples were taken from the 72 dewatered and composted biosolids amended plots. Samples were taken in November after each year's (2006 and 2007) crop harvest. Total heavy metal values were determined and compared across the application rates and between biosolids types. The effect of repeated applications of dewatered and composted biosolids was also evaluated and results of the quantitative changes in heavy metal concentrations in biosolids amended soil are presented below.

Comparison of total metals in soil and biosolids

The XRF results for total metals in the soil and biosolids at the beginning of 2006 is shown in Appendix B Table B.7. Here it can be seen that dewatered biosolids had significantly higher concentrations of total Cu and Zn than composted biosolids, whereas the concentration of Pb in composted biosolids was higher than the levels found in either the soil or the dewatered biosolids. However, the soil background concentrations of Mn and Ni were relatively similar with the levels found in both biosolids types. The amount of Fe in the soil and composted biosolids was similar and lower in the dewatered biosolids. It is expected that total Cu and Zn would accumulate noticeably with each application rate whereas there should be little difference in Mn, Ni or Fe. A possible decrease in Fe might be expected with the application of dewatered biosolids. Lead should increase slightly with the application of biosolids.

Table 5.1 XRF determined concentrations of total heavy metals in dewatered biosolids amended soil in canola plots in 2006 and 2007 (mg/kg).

Concentrations of total metals in dewatered biosolids amended soil in canola plots, 2006			
Biosolids rates (t/ha)	Cu	Zn	Ni
0	14 ± 2	30 ± 1	26 ± 1
5	15 ± 1	33 ± 1	25 ± 3
25	19 ± 1	37 ± 1	25 ± 1
45	26 ± 3	51 ± 4	27 ± 2
65	30 ± 2	55 ± 1	29 ± 3
Fertilized	16 ± 1	30 ± 1	25 ± 3
$LSD_{0.05}$	3.1***	3.8***	ns
Concentrations of total metals in dewatered biosolids amended soil in canola plots, 2007			
Biosolids rates (t/ha)	Cu	Zn	Ni
0	19 ± 1	40.1 ± 0.3	35.0 ± 0.1
5	23 ± 1	45 ± 3	36 ± 2
25	33 ± 3	63 ± 7	36 ± 4
45	43 ± 4	74 ± 6	37 ± 2
65	45 ± 5	82 ± 8	34 ± 1
Fertilized	20 ± 3	41 ± 4	36 ± 2
$LSD_{0.05}$	6.6***	11.5***	ns

The superscripts ***, * and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.001$, $p < 0.05$ and not significant respectively, $LSD_{0.05}$ stands for least significant difference (t-test) between mean values at $p < 0.05$ level. Values indicate means ± standard deviations of triplicate measurements (n = 3).

Total metal residues following application of dewatered biosolids

The effect of incorporating dewatered biosolids on the levels of total heavy metal residues in canola and oats plots is shown in Tables 5.1 and 5.2 below, and Table 5.3 summarizes the percentage changes in total metal levels due to dewatered biosolids applications

Table 5.2 XRF determined concentrations of total heavy metals in dewatered biosolids amended soil in oats plots in 2006 and 2007 expressed in mg/kg

Concentrations of total metals in dewatered biosolids amended soil in oats plots, 2006			
Biosolids rates (t/ha)	Cu	Zn	Ni
0	14 ± 1	31 ± 1	24 ± 1
5	15 ± 3	32 ± 2	25 ± 4
25	21 ± 3	42 ± 7	25 ± 2
45	25 ± 2	46 ± 2	28 ± 2
65	25 ± 1	47 ± 5	25 ± 5
F	15 ± 2	31 ± 1	25 ± 2
LSD $_{0.05}$	4.14***	8.3**	ns
Concentrations of total metals in dewatered biosolids amended oats plots, 2007			
Biosolids rates (t/ha)	Cu	Zn	Ni
0	18 ± 2	37 ± 1	36 ± 2
5	23 ± 2	46 ± 4	37 ± 3
25	30 ± 4	59 ± 8	35 ± 2
45	47 ± 2	88 ± 3	38.2 ± 0.1
65	49 ± 7	90 ± 9	37 ± 2
Fertilized	19 ± 3	39 ± 3	37 ± 2
LSD $_{0.05}$	7.2***	11***	ns

The superscripts ***, * and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.001$, $p < 0.05$ and not significant respectively, LSD $_{0.05}$ stands for least significant difference (t-test) between mean values at $p < 0.05$ level. Values indicate means ± standard deviations of triplicate measurements (n = 3).

Table 5.3 Changes in XRF determined total soil heavy metals in dewatered biosolids amended canola and oats plots in 2006 and 2007 experiments (mg/kg).

Dewatered biosolids treated canola plots				Dewatered biosolids treated oats plots		
Heavy metals	Unamended plot	Amended at 65 t/ha	Increases (in %)	Unamended plot	Amended at 65 t/ha	Increases (in %)
Cu Yr 1	14	30	114	14	25	79
Yr 2	19	45	137	18	49	172
Zn Yr 1	30	55	83	31	47	52
Yr 2	40	82	105	37	90	143

Year 1

In the 2006 experiment, at the highest dewatered biosolids receiving canola plots, biosolids application significantly increased the concentration of total heavy metals by 114 % for Cu and 83 % for Zn respectively (Table 5.3). As dewatered biosolids application rates increased, Cu and Zn levels in the amended soil showed linear increase with coefficient of determination values R^2 = 0.99 and R^2 =0.96, respectively (Table 5.4), however Ni levels were not significantly different from the unamended control plots.

Similarly, in the oats plots, 79% and 52% increase in Cu and Zn following dewatered biosolids applications were observed with coefficient of determination values of R^2 = 0.90, R^2 = 0.90 and R^2 = 0.95, respectively but there was no significant changes in Ni concentrations (Table 5.3 and 5.4).

Year 2

In 2007 experiment, compared with the unamended control plots, biosolids applications increased the level of total Cu and Zn by 137 and 105 % at the 65 t/ha dewatered biosolids treated canola plots. Concentration of Cu and Zn values in canola plots showed increasing trend with R^2 = 0.95 and R^2 = 0.97 respectively but Ni levels did not significantly change compared with the control plots (Table 5.3 and 5.4 and Appendix C).

Increases in Cu and Zn residuals in 2007 take into account the combined effect of the two years repeated dewatered biosolids applications.

In the oat plots, the changes were even greater with Cu and Zn levels increasing by 172 and 143 %, respectively. Concentrations of Cu and Zn in dewatered biosolids amended oats plots showed linear increases with R^2 = 0.95 and R^2 = 0.94 respectively (Table 5.3 and 5.4).

Significant differences ($p < 0.05$) in the mean Cu and Zn values between the two years experiment were also observed indicating that repeated dewatered biosolids applications had significant effect in changing total Cu and Zn levels in biosolids amended soil.

Regression of residual concentration against application rates

The impact of loading various biosolids application rates on heavy metals residues was quantified using regression analysis of the 2006 and 2007 data and presented in Table 5.4.

The analysis showed that in the 2006 experiment a one tonne application of dewatered biosolids increased the level of Cu and Zn on average by 0.25 and 0.40 mg/kg in canola plots and by 0.18 and 0.26 mg/kg in oats plots respectively, whereas in the 2007 experiment after repeated applications, loading one tonne of dewatered biosolids resulted in increases of Cu and Zn values by 0.20 and 0.32 mg/kg in canola plots and by 0.25 and 0.43 mg/kg in oats plots, respectively. The increase in the residuals of Cu and Zn in oats plots following dewatered biosolids application was greater than the observed increments in canola plots. The 2007 data on concentrations of Cu and Zn residuals indicates the combined effect of two years successive application of the biosolids.

Table 5.4 The impact of dewatered biosolids application rates on the level of total Cu and Zn, in amended soils for samples taken from canola and oats plots in 2006 and 2007 field experiments, a regression analysis.

Analytes	Amended soil from Canola plots			Amended soil from oats plots		
	Intercept	Regression coefficients	R^2	Intercept	Regression coefficients	R^2
Cu 2006	13.7***	0.25***	0.99	14.8**	0.18*	0.90
2007	21**	0.20**	0.95	19.4**	0.25**	0.95
Zn 2006	30***	0.40**	0.96	32***	0.26*	0.90
2007	43***	0.32**	0.97	40**	0.43**	0.94

The estimated values for the intercept and regression coefficients were tested whether the values were statistically different from zero or not, thus the superscripts *, **, *** and ns refer to significant levels (t-test) for the intercepts and regression coefficients at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively. The 2007 regression coefficients for Cu and Zn take into account the residuals remaining in the soil after two years successive application of dewatered biosolids. The mean values of triplicate measurements were plotted against application rates.

Effect of composted biosolids applications on XRF total heavy metals

The influence of composted biosolids applications on the concentrations of total heavy metals residue remained in the amended soil is shown in Table 5.5 and 5.6)

Year 1

When the control and the highest composted biosolids application rate (70 t/ha) were compared, total soil Cu and Zn, values in the 2006 composted biosolids treated canola plots increased significantly by 61 and 79 %, respectively, whereas, in the oats plots treated with composted biosolids, Cu and Zn values increased by 100 and 139 %, respectively (Table 5.5 and 5.6).

Year 2

Likewise, in the second year of the experiment, repeated application of composted biosolids in canola plots increased the concentration of total soil Cu and Zn by 153 and 195 % while in the oats plots Cu and Zn values showed a 240 and 334 % increases respectively (Table 5.5 and 5.6).

Table 5.5 XRF determined concentrations of total heavy metals in composted biosolids amended soil in canola plots in 2006 and 2007 expressed in mg/kg

Concentrations of total metals in composted biosolids amended soil in canola plots, 2006			
Biosolids rates (t/ha)	Cu	Zn	Ni
0	15 ± 2	33 ± 1	25.7 ± 0.1
10	15 ± 3	34 ± 4	25.3 ± 0.5
30	18 ± 2	44 ± 4	24.0 ± 0.6
50	23.6 ± 0.3	55 ± 4	24.4 ± 0.3
70	24.2 ± 0.5	59 ± 2	25 ± 1
Fertilized	14 ± 1	30 ± 1	25.0 ± 0.4
$LSD_{0.05}$	3.14***	7***	1.1*
Concentrations of total metals in composted biosolids amended soil in canola plots, 2007			
Biosolids rates (t/ha)	Cu	Zn	Ni
0	19 ± 1	41 ± 2	34 ± 1
10	22 ± 5	51 ± 8	34 ± 3
30	30 ± 2	71 ± 4	34 ± 1
50	40 ± 2	98 ± 4	37.0 ± 0.3
70	48 ± 3	121 ± 4	36 ± 2
Fertilized	19 ± 3	40 ± 1	34 ± 2
$LSD_{0.05}$	5.8***	9.8***	ns

The superscripts ***, * and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.001$, $p < 0.05$ and not significant respectively, $LSD_{0.05}$ stands for least significant difference (t-test) between mean values at $p < 0.05$ level. Values indicate means ± standard deviations of triplicate measurements (n = 3).

Table 5.6 XRF determined concentrations of total heavy metals in composted biosolids amended soil in oats plots in 2006 and 2007 (mg/kg).

Concentrations of total metals in composted biosolids amended soil in oats plots, 2006			
Biosolids rates (t/ha)	Cu	Zn	Ni
0	17 ± 3	38 ± 3	29 ± 6
10	19 ± 1	44 ± 2	30 ± 5
30	24 ± 3	60 ± 13	30 ± 6
50	28 ± 3	73 ± 15	32 ± 5
70	34 ± 8	91 ± 24	30 ± 6
Fertilized	16 ± 4	36 ± 5	29 ± 6
$LSD_{0.05}$	5.8***	22***	ns
Concentrations of total metals in composted biosolids amended soil in oats plots, 2007			
Biosolids rates (t/ha)	**Cu**	**Zn**	**Ni**
0	15 ± 1	32 ± 3	25 ± 1
10	21 ± 2	52 ± 2	36 ± 1
30	30 ± 1	71 ± 8	34 ± 1
50	42 ± 1	112 ± 3	35 ± 1
70	51 ± 1	139 ± 5	36 ± 1
Fertilized	18 ± 1	38 ± 1	34.0± 0.3
$LSD_{0.05}$	2.0***	9.9***	2***

The superscripts ***, * and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.001$, $p < 0.05$ and not significant respectively, $LSD_{0.05}$ stands for least significant difference (t-test) between mean values at $p < 0.05$ level. Values indicate means ± standard deviations of triplicate measurements (n = 3).

Table 5.7 Changes in XRF determined total soil heavy metals in composted biosolids amended canola and oats plots in 2006 and 2007 experiments.

Composted biosolids treated canola plots				Composted biosolids treated oats plots		
Heavy metals	Unamended plot (mg/kg)	Amended at 65 t/ha	Increase (in %)	Unamended plot	Amended at 65 t/ha	Increase (in %)
Cu Yr 1	15	24	61	17	34	100
Yr 2	19	48	153	15	51	240
Zn Yr 1	33	59	79	38	91	139
Yr 2	41	121	195	32	139	334

Regression of residual concentration against application rates

Total heavy metal analytical data generated from the 2006 and 2007 composted biosolids treated experiments were also subjected to regression analysis and results of the 2006 experiments showed that a one tonne application of composted biosolids in the canola plots increased the concentration of total soil Cu and Zn by 0.15 and 0.40 mg/kg respectively (Table 5.8). Similarly, Cu and Zn values in the oats plots increased by 0.24 and 0.75 mg/kg due to a one tonne application of composted biosolids.

In 2007, after repeated application, a one tonne of composted biosolids loading increased the levels of Cu and Zn residuals in canola plots by 0.21 and 0.58 mg/kg, whereas Cu and levels in oats plots increased by 0.26 and 0.76 mg/kg respectively (Table 5.8).

Table 5.8 The impact of composted biosolids application rates on the level of total Cu and Zn in amended soils samples taken from canola and oats plots in 2006 and 2007 experiment, a regression analysis.

Analytes	Amended soil from Canola plots			Amended soil from oats plots		
	Intercept	Regression coefficients	R^2	Intercept	Regression coefficients	R^2
Cu 2006	14**	0.15**	0.93	17***	0.24***	0.99
2007	18**	0.21***	0.99	15***	0.26***	0.99
Zn 2006	32***	0.40**	0.97	37***	0.75***	0.99
2007	40***	0.58***	0.99	33***	0.76***	0.99

The estimated values for the intercept and regression coefficients were tested whether the values were statistically different from zero or not, thus the superscripts *, **, *** and ns refer to significant levels (t-test) for the intercepts and regression coefficients at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively. The 2007 regression coeffients for Cu and Zn take into account the residuals remaining in the soil after two years successive application of composted biosolids.

For soil samples taken from oat plots treated with composted biosolids, the regression coefficients for Cu and Zn were significantly higher than the corresponding values for canola plots, indicating that greater concentrations of Cu and Zn residues were accumulated in the oats plots than in the canola plots.

5.2. DTPA extractable heavy metals

After each year's successive application of biosolids, the bioavailable fractions of heavy metals residues remained in dewatered and composted biosolids amended soils were extracted using DTPA procedure and quantified by ICP-MS.

5.2.1. Analytical Quality assurance

To validate the analytical data of the DTPA extractable metals, a multi element stock certified standard solution containing the heavy metals were analysed in duplicate for every ten samples and the percentage recovery was calculated.

The measured and certified values of DTPA extractable heavy metals as described in Table 6.9 below indicated that the recovery of the laboratory check sample (Multi-element stock ICP-MS solution) ranged between 99 % for Pb and 101 % for Fe, respectively.

Table 5.9 Results of laboratory control samples (ICP-MS multi-element stock solution) analysed in duplicate for every 10 batch of biosolids amended soil samples to validate the data for DTPA extractable heavy metals.

Analyses	Cu	Zn	Mn	Fe	Co	Ni	Pb
Measured	99.5	98	99 ± 1	9957	99 ± 1	98 ± 1	99 ± 1
Certified	99	99	99	9900	99	99	99
Recovery (%)	100	99	100	101	100	99	99

5.2.2. DTPA extractable heavy metals in dewatered and composted biosolids amended soil

At the beginning of the experiment, soil and dewatered biosolids and composted biosolids samples were analysed for DTPA extractable heavy metal concentrations. As shown in Table 5.10, the levels of Cu, Zn, Mn and Ni in dewatered biosolids were significantly higher than the levels found in composted biosolids; however the concentrations of Fe and Pb in composted biosolids were slightly higher than those found in dewatered biosolids. For all of the metal concentrations, the soil background concentrations were lower than the biosolids.

Table 5.10 Results of concentrations of DTPA extractable heavy metals in soil, dewatered biosolids and composted biosolids (mg/kg).

Analytes	Soil	Dewatered biosolids	Composted biosolids
Heavy metals extracted using DTPA and analysed by ICP-MS (mg/kg).			
Cu	1.1 ± 0.1	185 ± 32	22 ± 4
Zn	0.9 ± 0.3	368 ± 3	271 ± 57
Mn	12 ± 1	98 ± 3	28 ± 5
Fe	82.00 ± 0.01	208 ± 3	270 ± 62
Co	0.2 ± 0.02	0.70 ± 0.03	0.24 ±0.06
Ni	0.94 ± 0.06	4.8 ± 0.3	1.6 ± 0.4
Pb	0.54 ± 0.34	4.9 ± 0.01	9 ± 2

Effect of dewatered biosolids applications on DTPA extractable heavy metals

Over the two years of the study, dewatered biosolids at all application rates significantly increased the bioavailable fractions of Cu, Zn, Mn and Fe levels in amended soils in which canola and oats were grown (Fig. 5.1 and 5.2).

Year 1

In the 2006 experiment, when the control and the highest (65 t/ha) biosolids receiving plots were compared, DTPA extractable Cu and Zn fractions increased by 275 % and 171 % in canola plots and by 221 % and 507 % in oats plots respectively.
The concentrations of Mn and Fe fractions in the 2006 experiment increased by 39 % and 56 % in canola plots and by 11% and 116 % in oats plots respectively.
In the 2006 experiment, compared with the control plots, Co, Ni and Pb increased by 100%, 16% and 36 % in canola plots and Ni and Pb increased by 21% and 57 % in oats plots, however Co levels in the 2006 oats plots were not significantly different from the control plots (Table 5.11).

Year 2

In 2007, reapplying biosolids the same rates as in 2006, Cu and Zn fractions at the 65 t/ha application rates increased by 3.5 and 11 folds in canola plots and increased by 5 and 16 folds in oats plots compared with the control plots (Fig 5.1).

The levels of Cu and Zn in the 2007 biosolids amended canola and oats plots were significantly different ($p < 0.05$) from the levels found in the 2006 recordings.

In the 2007 experiment, when Mn and Fe levels at the highest (65 t/ha) biosolids receiving canola plots were compared with their corresponding levels recorded in the 2006 canola plots, Mn and Fe levels increased by 73 % and 189 % respectively (Fig.5.1 and 5.2); whereas Mn and Fe levels in oats plots increased by 63 % and 130 % respectively (Table 5.11).

The results also showed that as dewatered biosolids application rates increased, extractable Cu, Zn, Mn and Fe also showed significant linear increments for both crops in both years of the experiment. Extractable heavy metal concentrations in the conventionally fertilized plots were not significantly different from the control plots (Fig. 5.1 and 5.2).

Biosolids loading rates had significant effect in increasing the DTPA extractable Co, Ni and Pb in amended soil in both canola and oats plots during the two years period of study, with the exception of Co in 2006 oats plots and Pb in 2007 canola plots which were not significantly different from the control plots (Fig.5.3 and 5.4).

DTPA extractable Co, Ni and Pb also showed upward trend following dewatered biosolids application rates in both years of the experiment.

When the 2007 and 2006 results of the 65 t/ha biosolids treated plots were compared, the increase in Ni and Pb levels in 2007 was significantly higher than the levels recorded in 2006 for both crops (Table 5.11).

DTPA extractable Co, Ni and Pb also showed linear increments following biosolids application rates in both years of the experiment.

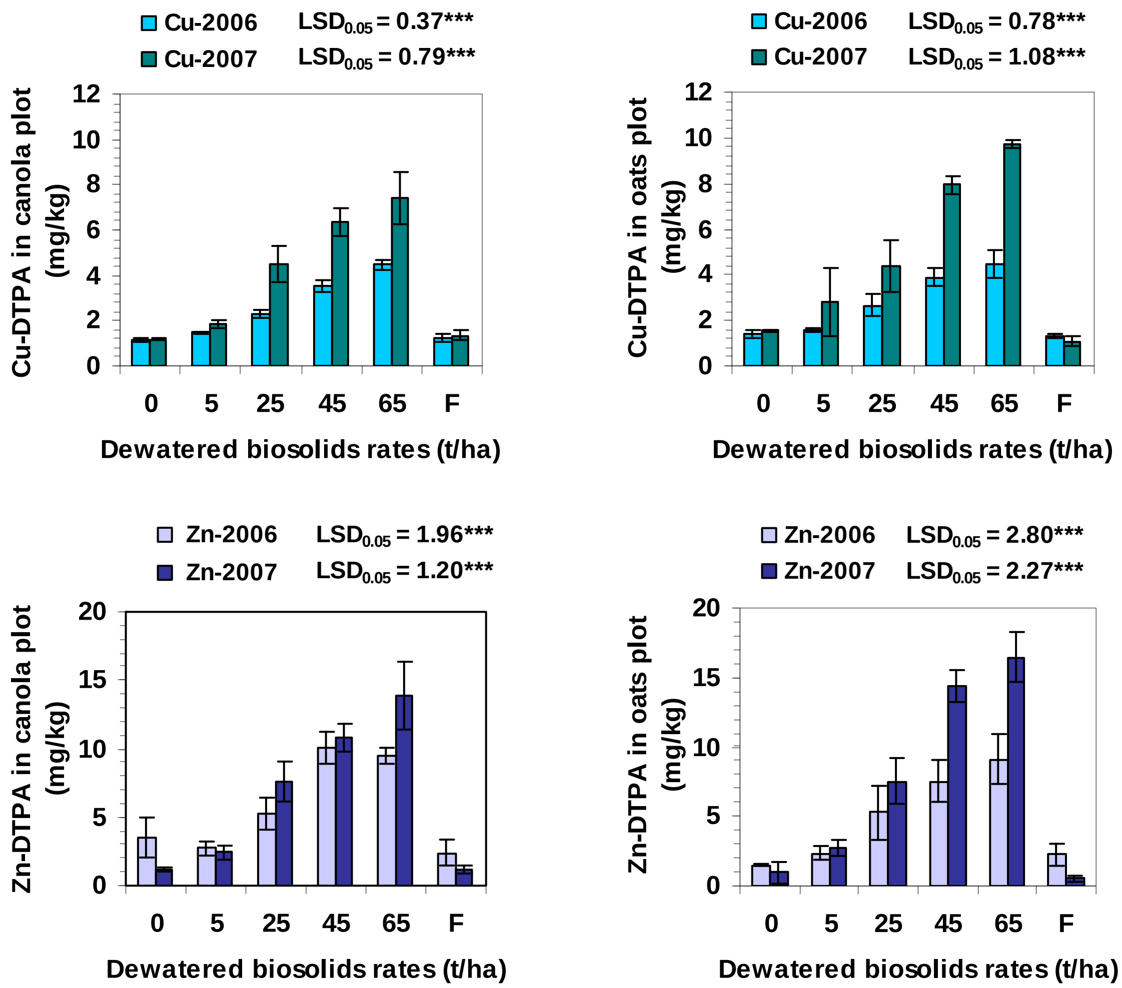


Figure 5.1 Effect of loading various dewatered biosolids application rates on DTPA extractable Cu and Zn concentrations in amended clay loam soils from canola and oat plots in 2006 and 2007 field experiments

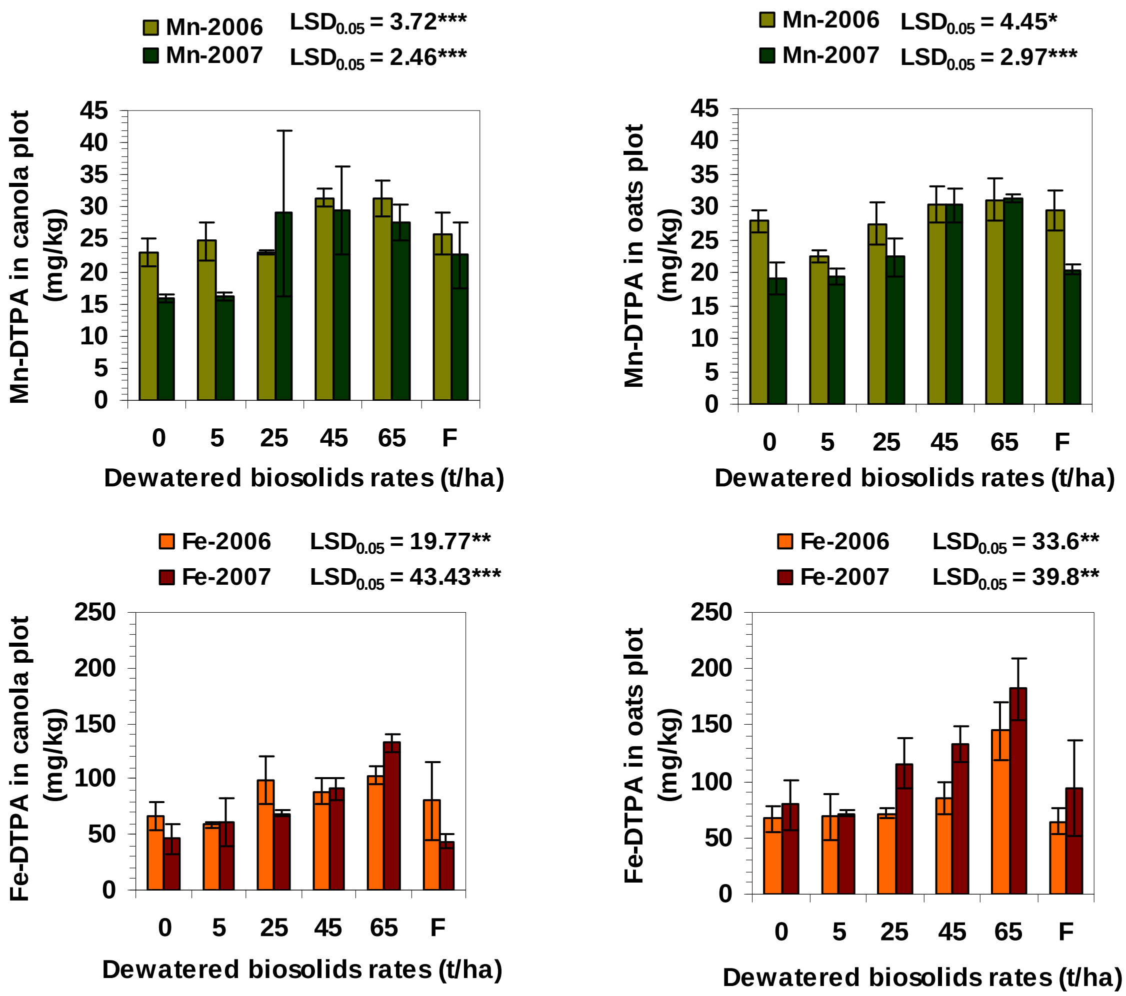


Figure 5.2 Effect of loading various dewatered biosolids application rates on DTPA extractable Mn and Fe levels in amended clay loam soils from canola and oat plots in 2006 and 2007 field experiments. The error bars indicate standard deviations of triplicate measurements. LSD $_{0.05}$ refers to the least significant difference (t-test) between mean values at 5% probability level. Whereas, *,**,*** and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$,$p < 0.001$ and not significant respectively(n = 3).

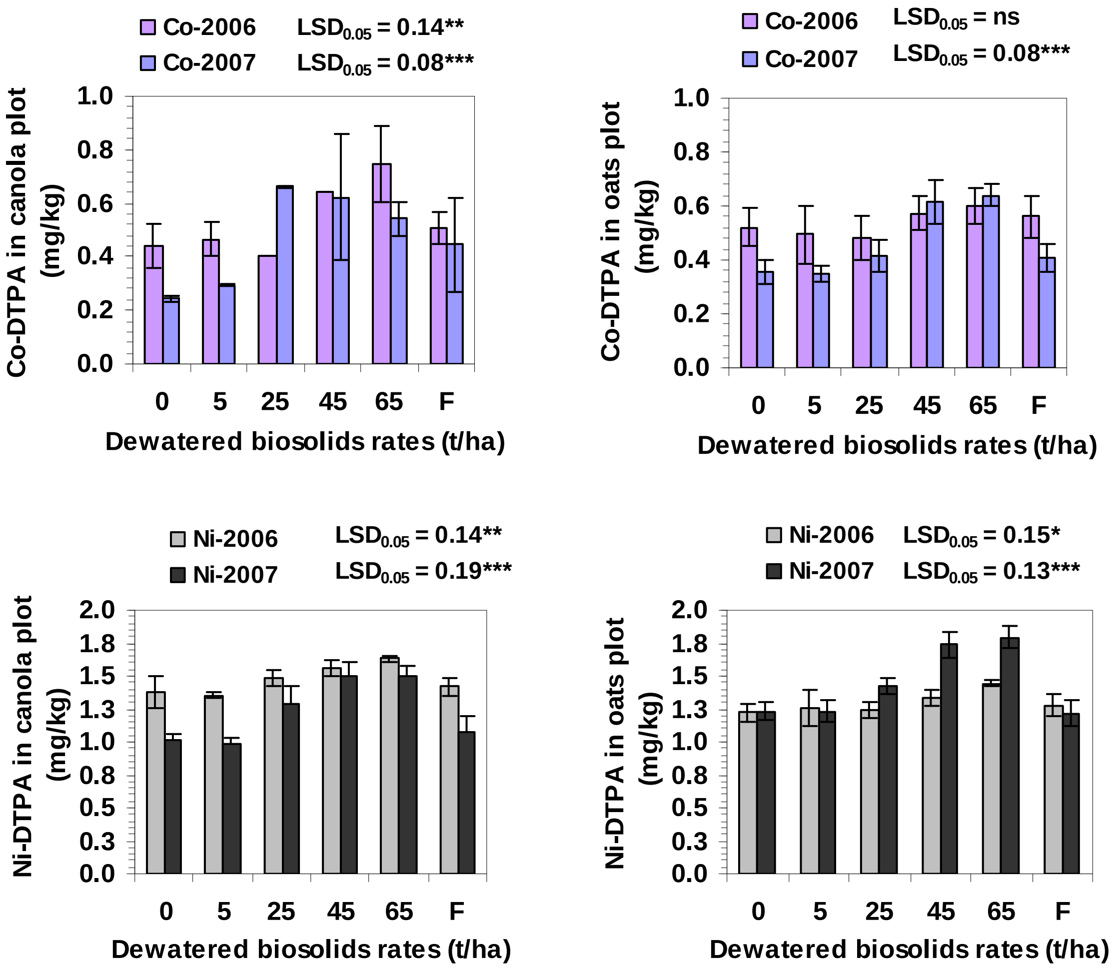
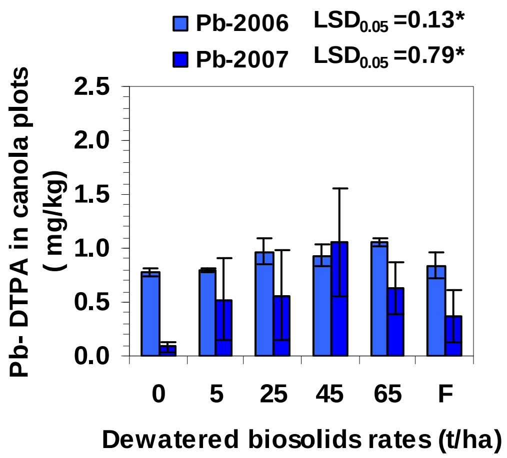


Figure 5.3 Effect of loading various dewatered biosolids application rates on DTPA extractable Co and Ni levels in amended clay loam soils from canola and oat plots in 2006 and 2007 field experiments. The error bars indicate standard deviations of triplicate measurements. LSD $_{0.05}$ refers to the least significant difference (t-test) between mean values at 5 % probability level. Whereas, *,**,*** and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively ($n = 3$).



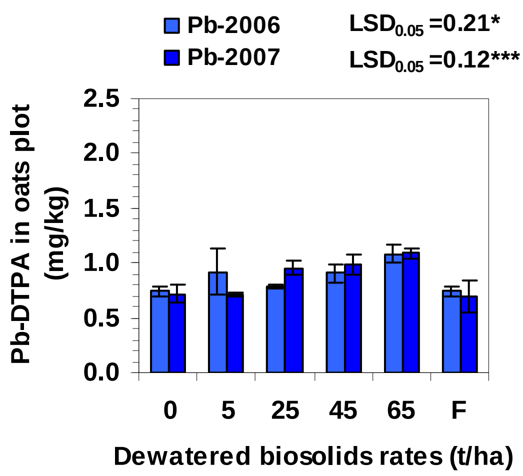


Figure 5.4 Effect of loading various dewatered biosolids application rates on DTPA extractable Pb levels in amended clay loam soils from canola and oat plots in 2006 and 2007 field experiments. The error bars indicate standard deviations of triplicate measurements, whereas The LSD $_{0.05}$ refers to the least significant difference (t-test) between mean values at 5 % probability level, whereas, *,**,*** and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively (n = 3).

Table 5.11 Changes in DTPA extractable soil heavy metals in dewatered biosolids amended canola and oats plots in 2006 and 2007 experiments.

Dewatered biosolids treated canola plots				Dewatered biosolids treated oats plots		
Heavy metals	Unamended plot (mg/kg)	Amended at 65 t/ha (mg/kg)	Changes relative to unamended plot (in %)	Unamended plot	Amended at 65 t/ha	Changes relative to unamended plot (in %)
Cu Yr 1	1.2	4.5	275	1.4	4.5	221
Yr 2	1.8	8.11	351	1.54	9.7	530
Zn Yr 1	3.5	9.5	171	1.5	9.1	507
Yr 2	1.1	13.9	1164	0.95	16.5	1637
Mn Yr 1	23	32	39	28	31	11
Yr2	16	27.6	73	19.2	31.3	63
Fe Yr 1	66	103	56	67	145	116
Yr 2	46	133	189	79	182	130
Co Yr 1	0.4	0.8	100	0.5	0.6	20
Yr 2	0.24	0.54	125	0.36	0.64	78
Ni Yr 1	1.4	1.63	16	1.2	1.45	21
Yr 2	1.02	1.5	47	1.23	1.79	46
Pb Yr 1	0.77	1.05	36	0.7	1.1	57
Y 2	0.09	1.0	900	0.7	1.79	156

Effect of composted biosolids applications on DTPA extractable heavy metals, Mn and Fe

Year 1

The response of DTPA extractable Cu, Zn, Fe and Pb in the 2006 canola plots was linear and increased following composted biosolids applications. The concentrations of Cu, Zn and Fe recorded at the highest composted biosolids treatment (70 t/ha) when compared with the control plot increased by 1.1, 7.0 and 1.7 fold, respectively, whereas Pb increased by 56 %. However, Co, Ni and Mn levels were not different from the control plots (Table 5.12).
Likewise, in oats plots receiving composted biosolids in the 2006 experiment, Cu, Zn Fe, Co and Pb also showed linear response to increased rates of composted biosolids additions and the values increased by 2.8, 26, 1.6 fold for Cu, Zn and Fe respectively and Pb increased by 96 %, but, Mn and Ni showed no significant increases (Fig. 5.5, 5.7 and 5.8).

Year 2

Similarly, in 2007, DTPA extractable Cu, Zn and Pb values in canola plots showed a linear response to composted biosolids treatments and increased by 1.5, 9.0 and 1.4 folds respectively, whereas, Fe increased by 65%. Mn and Ni were not significantly different from the control plots.

In the same way, Cu, Zn, Fe and Pb in oats plots displayed a linear trend as composted biosolids rates increased; however, the increase in Mn, Co and Ni were not significant (Fig. 5.5, 5.7 and 5.8).

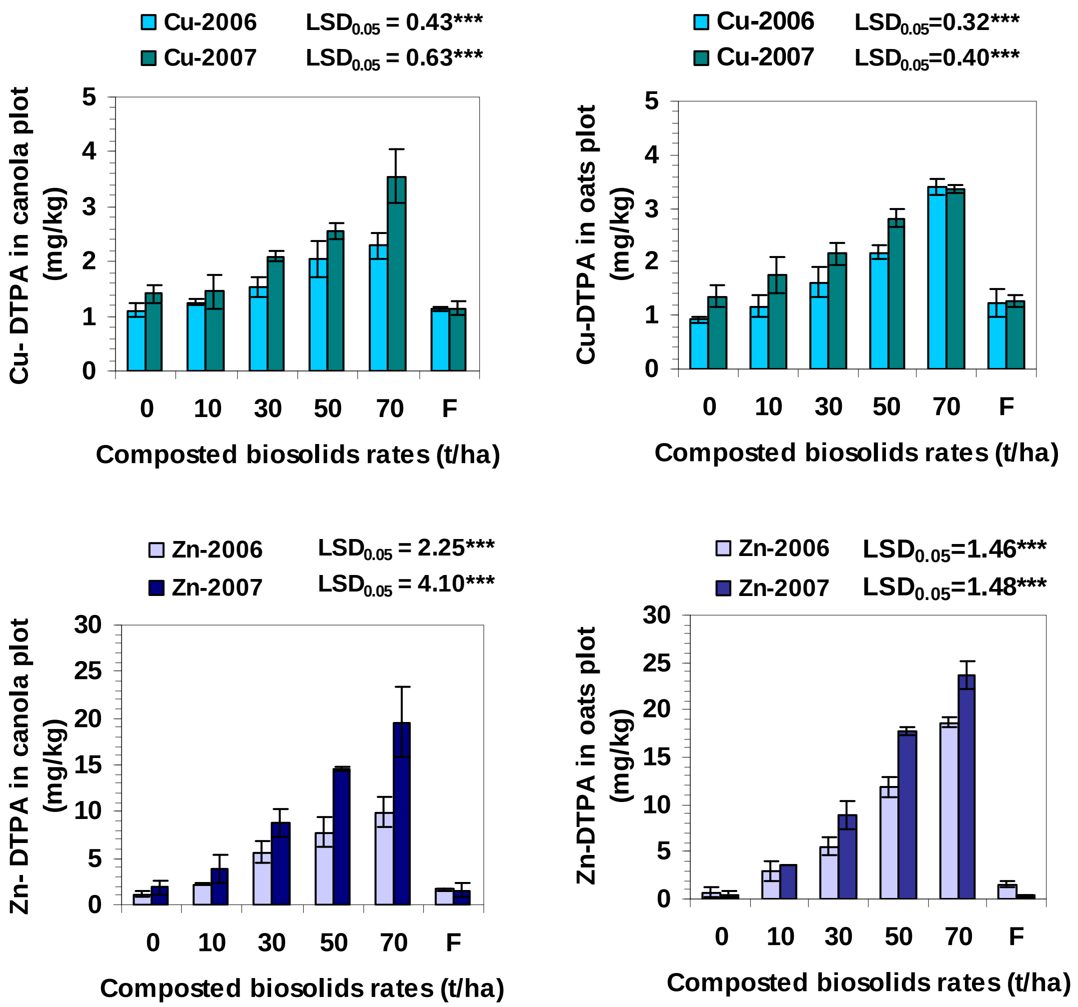


Figure 5.5 Effect of loading various composted biosolids application rates on DTPA extractable Cu and Zn levels in amended clay loam soils from canola and oat plots in 2006 and 2007 field experiments. The error bars indicate standard deviations of triplicate measurements. LSD $_{0.05}$ refers to the least significant difference (t-test) between mean values at 5 % probability level. Whereas, *,**,*** and ns refers to significant treatment effects in ANOVA (F-test) at p < 0.05, p <0 .01, p < 0.001 and not significant respectively (n = 3).

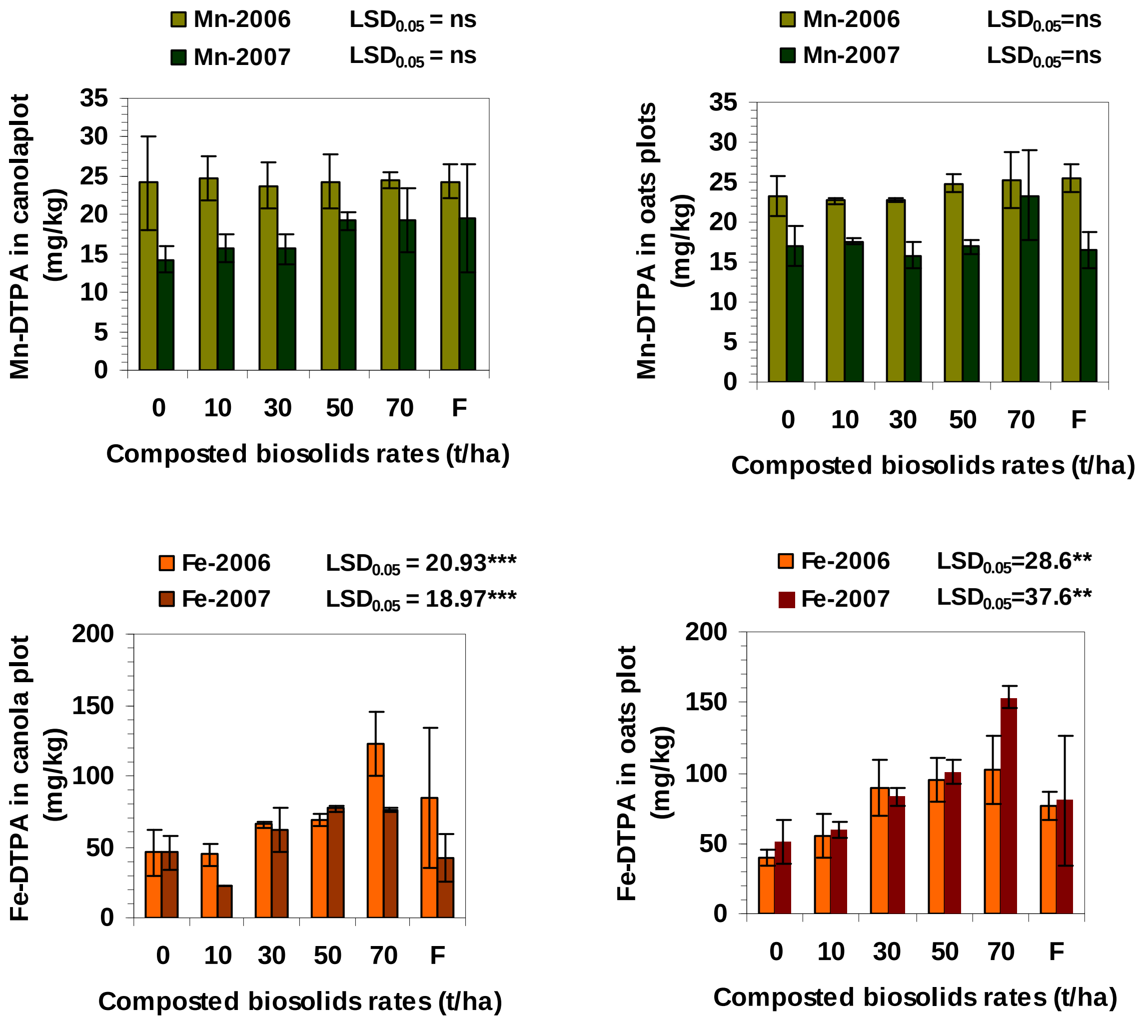


Figure 5.6 Effect of loading various composted biosolids application rates on DTPA extractable Mn and Fe levels in amended clay loam soils from canola and oat plots in 2006 and 2007 field experiments. The error bars indicate standard deviations of triplicate measurements. LSD $_{0.05}$ refers to the least significant difference (t-test) between mean values at 5 % probability level. Whereas, *,**,*** and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively (n = 3).

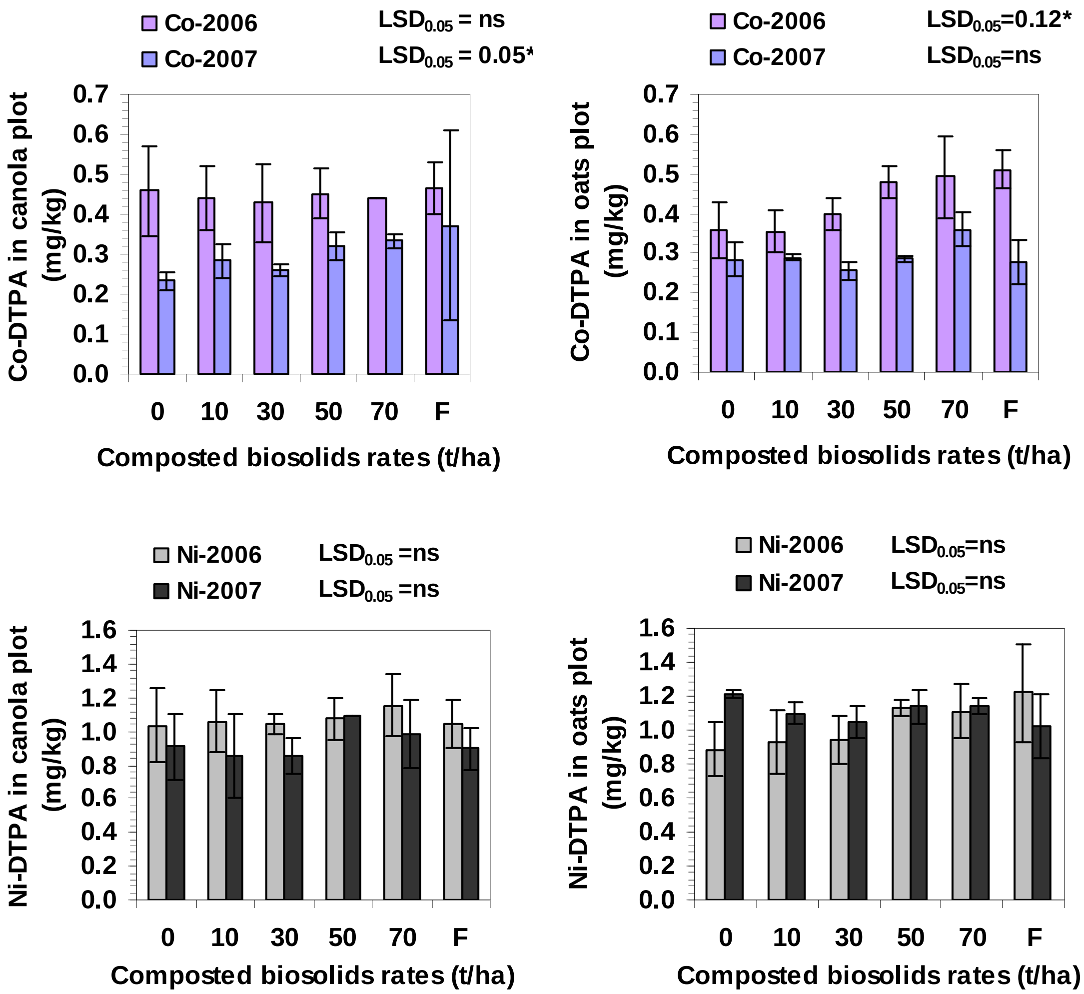


Figure 5.7 Effect of loading various composted biosolids application rates on DTPA extractable Co and Ni levels in amended clay loam soils from canola and oat plots in 2006 and 2007 field experiments. The error bars indicate standard deviations of triplicate measurements.

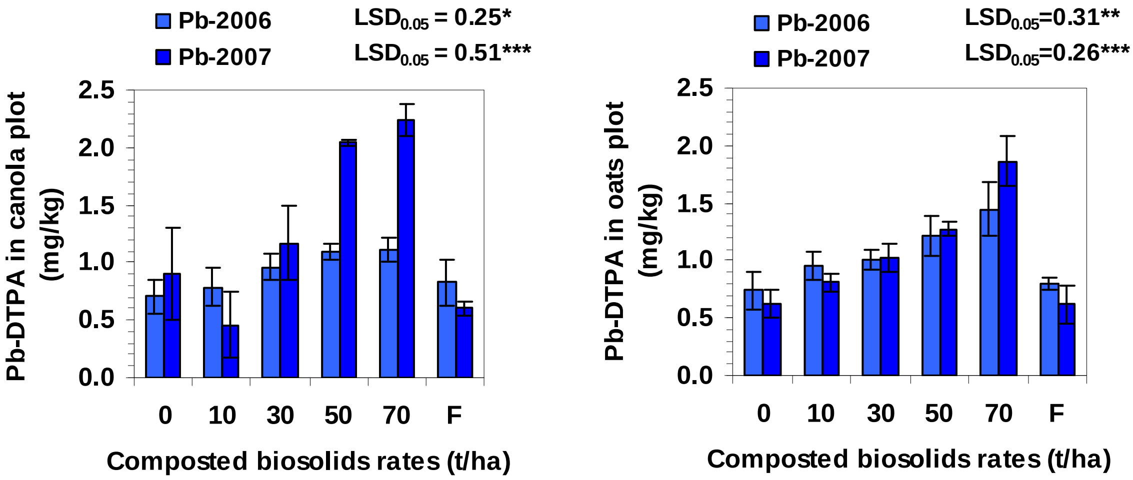


Figure 5.8 Effect of loading various composted biosolids application rates on DTPA extractable Pb levels in amended clay loam soils from canola and oats plots in 2006 and 2007 field experiments.

Table 5.12 Changes in DTPA extractable soil heavy metals in composted biosolids amended canola and oat plots in 2006 and 2007 experiments.

Composted biosolids treated canola plots				Composted biosolids treated oats plots		
Heavy metals	Unamended plot (mg/kg)	Amended at 65 t/ha (mg/kg)	Changes relative to unamended plot (in %)	Unamended plot	Amended at 65 t/ha	Changes relative to unamended plot (in %)
Cu Yr 1	1.1	2.3	109	0.9	3.4	278
Yr 2	1.4	3.6	157	1.4	3.4	143
Zn Yr 1	1.2	9.9	725	0.7	18.7	2571
Yr 2	2.0	20	900	0.5	23.7	4640
Mn Yr 1	24	25	4	23	25	9
Yr2	14	19	36	17	23	35
Fe Yr 1	46	123	167	40	102	155
Yr 2	46	76	65	51	154	202
Yr 2	0.9	1.0	11	1.21	1.14	-6
Pb Yr 1	0.7	1.1	57	0.7	1.5	114
Y 2	0.9	2.2	144	0.6	1.9	217

Discussion

In both years of the experiment increases in total soil Cu and Zn levels following dewatered biosolids and composted biosolids application rates were observed. Likewise, increases in total heavy metal levels due to biosolids applications was reported by Walter *et al.* (2002) using 50 and 100 t dry solids/ha anaerobically digested biosolids from two different waste treatment plants containing high levels of heavy metals. It was reported that biosolids application significantly ($p < 0.05$) increased the total concentrations of heavy metals, with the exception of Ni in the low biosolids treatments. Similarly, Mantovi *et al.* (2005) also evaluated the effect of 5 and 10 t dry solids/ha sewage sludge application on soil and crops (Wheat and maze) and reported that sewage sludge application lowered soil pH and increased the total Cu and Zn levels in the soil.

The levels of Cu and Zn recorded in oats plots treated with composted biosolids were higher than those found in canola plots in both years of the experiments. This indirectly indicates that canola may have extracted more biosolids Cu and Zn than oats.

In this study, the dewatered biosolids used at the beginning of the experiment had high concentrations of both total and DTPA extractable Cu, Zn, Mn, and Pb, hence significant levels of the metals were transferred from the biosolids to the soil.

In addition to this, the increase in the DTPA extractable fractions of metals in the biosolids amended soil could possibly be attributed due to the decrease in the pH of the soil following biosolids application and the solubility of the metal complexes from biosolids organic matter which in both cases resulted in the release of metals in soil and hence increasing the DTPA extractable pools.

The total concentration of Fe recorded in the background soil was higher than the levels found in the dewatered biosolids and composted biosolids, but, DTPA extractable Fe concentration in composted biosolids and dewatered biosolids was approximately 3 folds higher than the level of Fe found in the background soil and hence, as expected, Fe levels in biosolids amended soil increased as biosolids loading rates increased.

Sewage sludge properties on sludge-borne metal availability have a significant impact on metal availability in soil. The potential for metal release from sludges mainly depends upon sludge compositional factors (Merrington and McLaughlin, 2003).

In some instances Organic matter in sludges may bind metals; however such binding process could possibly be offset by the formation of soluble organo-metal complexes and the loss of complexation capacity through organic matter degradation (Karapanagiotis et al., 1991).

Thus, during ageing and organic matter decomposition, metals bound in humic substances, biomass and noncrystalline materials will presumably be released into the soil solution soil (Baldwin *et al*., 1983; Martinez *et al*., 2001). Because of microbial degradation of organic matter, organic binding sites are lost which would result in sludge borne metals becomming increasingly available (Hooda and Alloway 1994) and hence organic matter degradation is considered as one possible reason for the release of metals in sewage sludge amended soil (McBride, 1995; Sadovnikova *et al*., 1996).

Results of this study have shown that pH of the biosolids and soluble metals complexes contained in biosolids organic matter had a significant impact on metals solubility and hence their release to the amended soil (Fig 5.15).

To this end, repeated biosolids application did not pose any negative impact from heavy metals on the biosolids receiving soil since heavy metals levels did not exceed the EPA ceiling limits (EPA Vic., 2004).

Even though, the total heavy metal concentration in dewatered biosolids was higher than composted biosolids, higher levels of extractable Cu and Zn were noted in composted biosolids treated plots, this could be due to the composting process which increases the available fractions of metals in the composted biosolids amended plots. Composting increases the availability of Cu and Zn because of the contribution of microbial activity to the loss of exchangeable sites often found in organic matter during its decomposition (Garrido *et al.*, 2005; He *et al*., 1992). Indeed, Cu and Zn may be mobile during composting because of the direct correlation that both have with organic carbon (Darmody *et al*., 1983).

As shown in Fig. 5.6, the extractable Mn levels in soils after cropping in 2007 all showed a decrease compared to the previous year. This was particularly pronounced in the case of soils amended with composted biosolids. This phenomenon could have been due to a combination of speciation changes caused by the organic matter imputs and also leaching of complexed Mn from the top part of the soil profile.

5.3. Relationship between DTPA and XRF determined heavy metals

Copper

In the 2006 dewatered biosolids and composted biosolids treated canola plots, significant positive relationship between DTPA extractable (ICP-MS) and XRF determined total Cu concentrations were (R^2 = 0.93 and R^2 = 0.78) respectively . Similarly, in 2007 dewatered biosolids and composted biosolids treated canola plots concentrations of Cu were also positively related (R^2 = 0.87 and R^2 = 0.85) respectively (Fig. 5.9).

Likewise, the coefficient of determination values for Cu in the 2006 oat plots treated with dewatered biosolids and composted biosolids were all positive and significant (R^2 = 0.73 and R^2 = 0.90), however in the 2007 oats plots a stronger positive coefficient of determination values for Cu were noted (R^2 = 0.89 and R^2 = 0.95) (Fig. 5.9).

Zinc

Correspondingly, the coefficient of determination values for Zn in the 2006 dewatered biosolids and composted biosolids treated canola plots were R^2 = 0.88 and 0.79 respectively, and in the 2007 canola plots treated with dewatered biosolids and composted biosolids were R^2 = 0.78 and R^2 = 0.91). In the same way, the coefficient of determination values for the 2006 oats plots treated with dewatered biosolids and composted biosolids were R^2 = 0.55 and R^2 = 0.85, however in 2007 stronger relationship with coefficients of determination values (R^2 = 0.91 and R^2 = 0.98) were observed (Fig. 5.10)

The correlations coefficients for Mn and Fe determined using DTPA extraction (ICP-MS) and XRF were weak and not significant.

In general, the correlations between DTPA extractable (ICP-MS) and XRF determined Cu and Zn values in biosolids amended soil were strongly correlated, furthermore in 2007 after repeated application of dewatered and composted biosolids in both canola and oats plots stronger positive correlations were noted. Dewatered biosolids and composted biosolids had significantly higher levels of organic matter and heavy metals than the soil background concentration, thus significant correlations between DTPA extractable and XRF determined total heavy metal concentrations particularly for Cu and Zn in both canola and oats plots were noted.

As expected the data presented in Fig. 5.9 and 5.10 show that Cu extractability is lower in compost amended soil compared to digested sludge. Compost contained 60 % less Cu than digested cake and the results support the view that low metal content materials have intrinsically lower bioavailabilities than high metal materials at equivalent total soil concentrations (Smith, 2009). With Zn, on the other hand, both materials had similar contents and the extractable concentrations were broadly similar, albeit slightly smaller generally with compost which would be consistent with the generally lower availability of metals in this material compared to digested sludge.

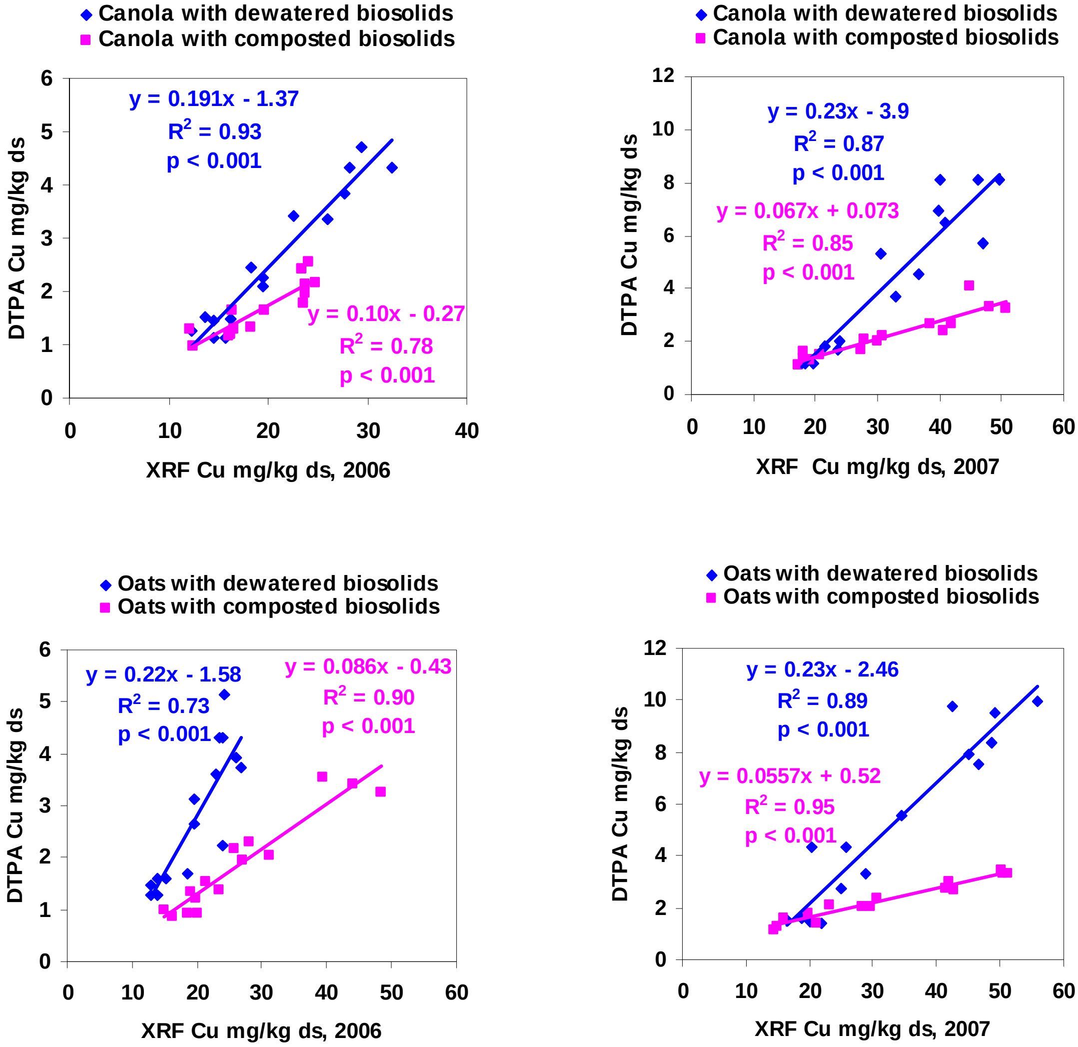


Figure 5.9 Relationship between DTPA extractable (ICP-MS) and XRF determined concentrations of Cu residuals in dewatered biosolids and composted biosolids amended soils samples taken from canola and oats plots after the end of 2006 and 2007 experiments. The probability value (p < 0.001 stands for the regression coefficient for the independent variable XRF determined total Cu concentration.

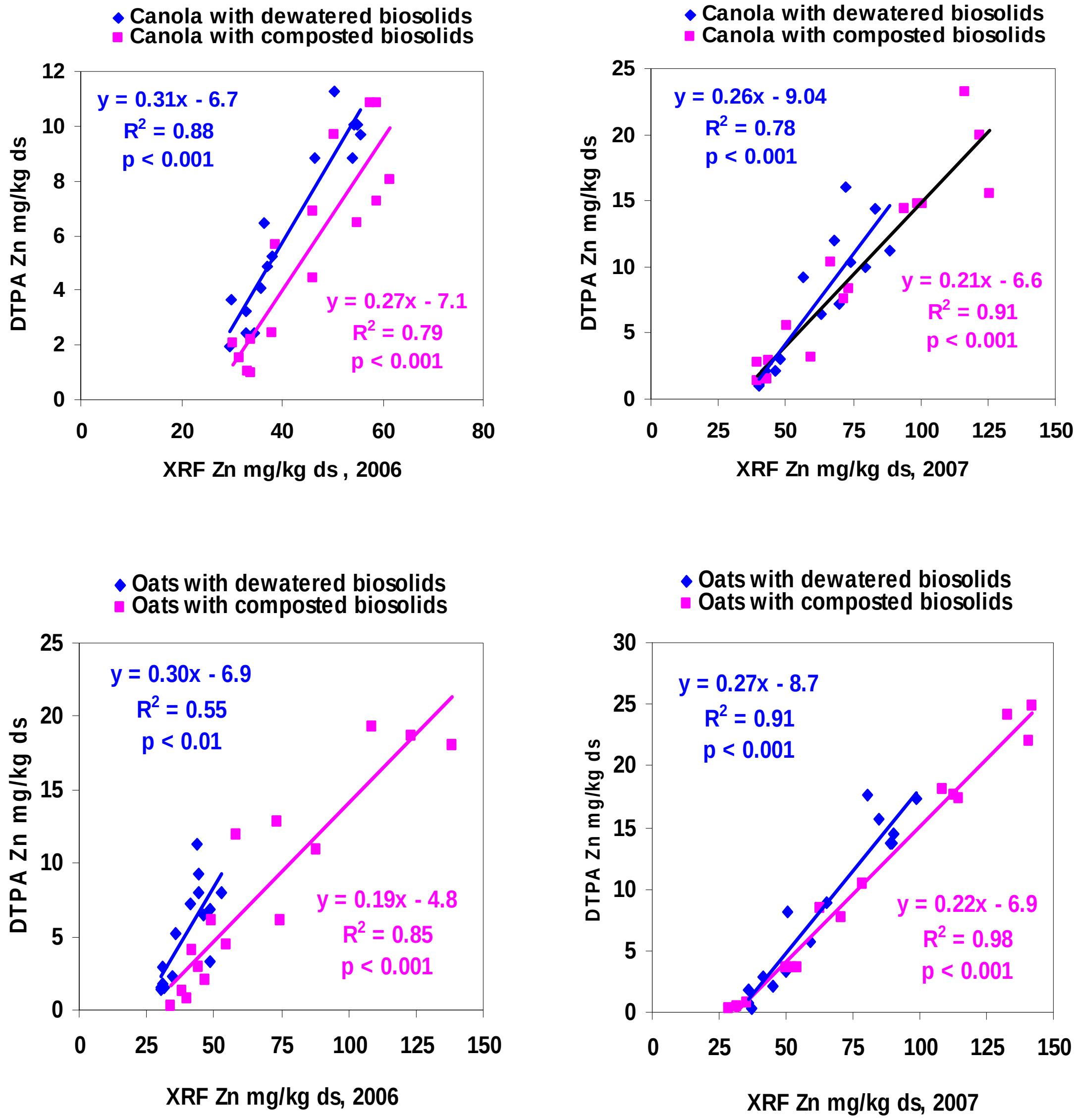


Figure 5.10 Relationship between DTPA extractable (ICP-MS) and XRF determined concentrations of Zn residuals in dewatered biosolids and composted biosolids amended soils samples taken from canola and oats plots after the end of 2006 and 2007 experiments. The probability value ($p < 0.001$ stands for the regression coefficient for the independent variable XRF determined total Zn concentration.

5.4. Changes in the ratio of DTPA to XRF determined soil Cu, Zn, Mn and Fe levels

To examine the effect of dewatered biosolids and composted biosolids application rates on the changes and trends of bioavailable fractions of heavy metals (extracted by DTPA,) the ratio of DTPA extractable and XRF concentrations of metals in biosolids amended soil samples were plotted against biosolids application rates. Table 5.13 shows the ratio of DTPA extractable to XRF determined total metals concentration in the background soil, dewatered biosolids and composted biosolids expressed as a percentage.

The values for the ratio of DTPA to XRF metals recorded in dewatered biosolids were higher than the corresponding values found in composted biosolids. The ratio for the soil background concentration was the lowest for all the heavy metals. The effects for each of the metals analysed is discussed below.

Table 5.13 The ratio of DTPA extractable to XRF determined total heavy metals values expressed in percent for background soil, dewatered biosolids and composted biosolids

Analytes	Soil	Dewatered biosolids	Composted biosolids
Cu	6.5	28.5	11.0
Zn	2.4	34.7	33.0
Mn	4.9	46.0	9.4
Fe	0.3	1.5	1.1
Ni	4.0	17.7	7.5
Pb	4.7	17.5	19.0

Concentrations of DTPA extractable and XRF total heavy metals were analysed before at the beginning of the experiment in 2006.

Cu and Zn

In the 2006 and 2007 dewatered biosolids treated canola and oats plots the ratio of DTPA to XRF determined soil Cu levels showed an increasing trend following dewatered biosolids application rates. The levels of DTPA extractable Cu in oats plots were relatively higher than the levels recorded in canola plots.

In contrast, the ratio of DTPA to XRF levels of Cu in composted biosolids treated canola and oats plots did not show an upward trend and was significantly lower than the levels found in dewatered biosolids amended canola and oats plots in both years of the experiment (Fig. 5.11).

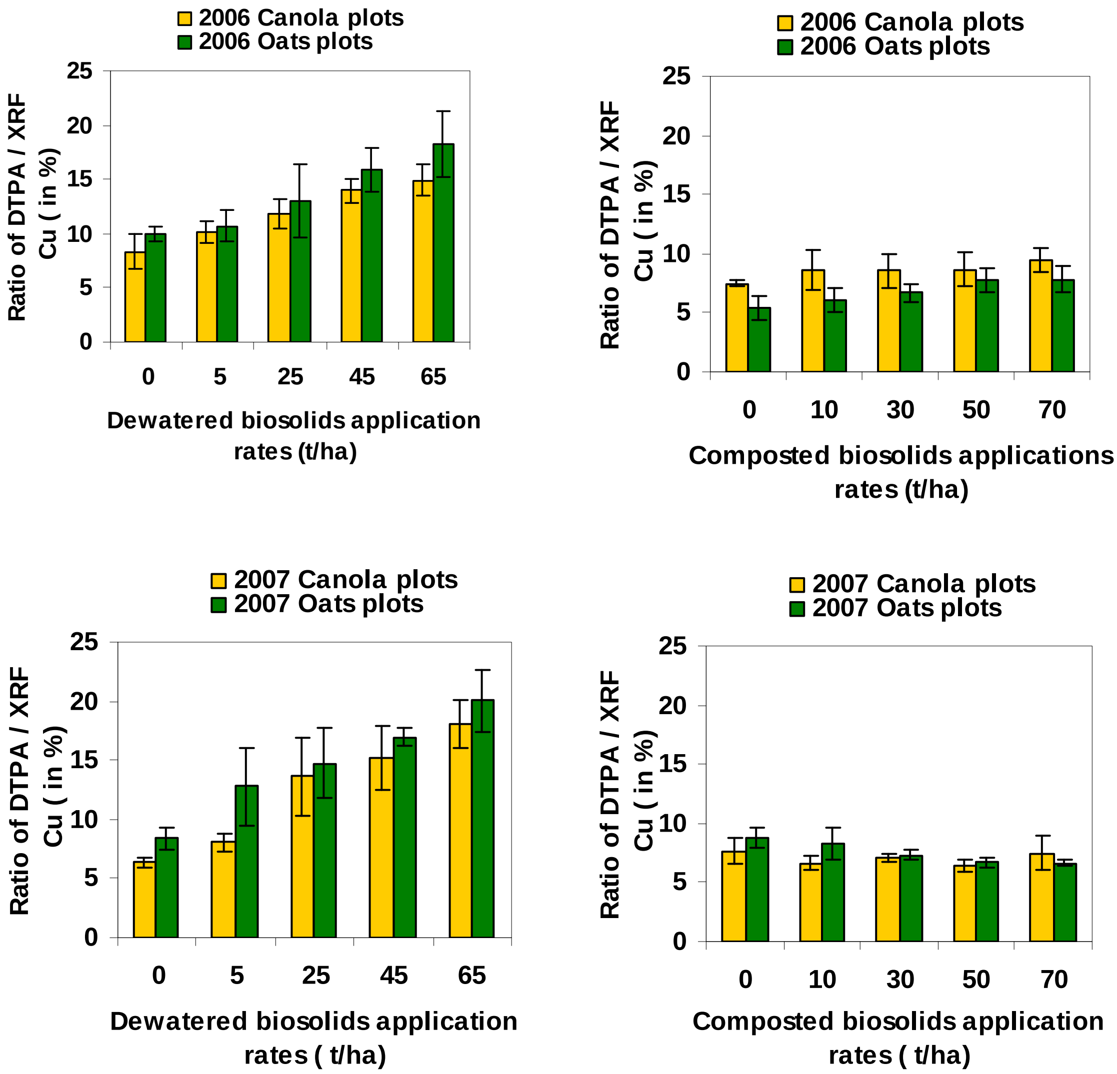


Figure 5.11 The influence of dewatered biosolids applications on the ratio of DTPA to XRF determined soil Cu levels in canola and oats plots in 2006 and 2007 experiments.

The ratio of DTPA extractable to XRF determined Zn values in dewatered biosolids and composted biosolids amended canola and oats plots showed better upward trend than Cu in both years of the experiments. The observed ratio of DTPA to XRF determined Zn values in dewatered and composted biosolids treated plots were relatively similar, however Zn ratio observed in oats plots treated with dewatered and composted biosolids were slightly higher than the corresponding values observed in canola plots (Fig. 5.12).

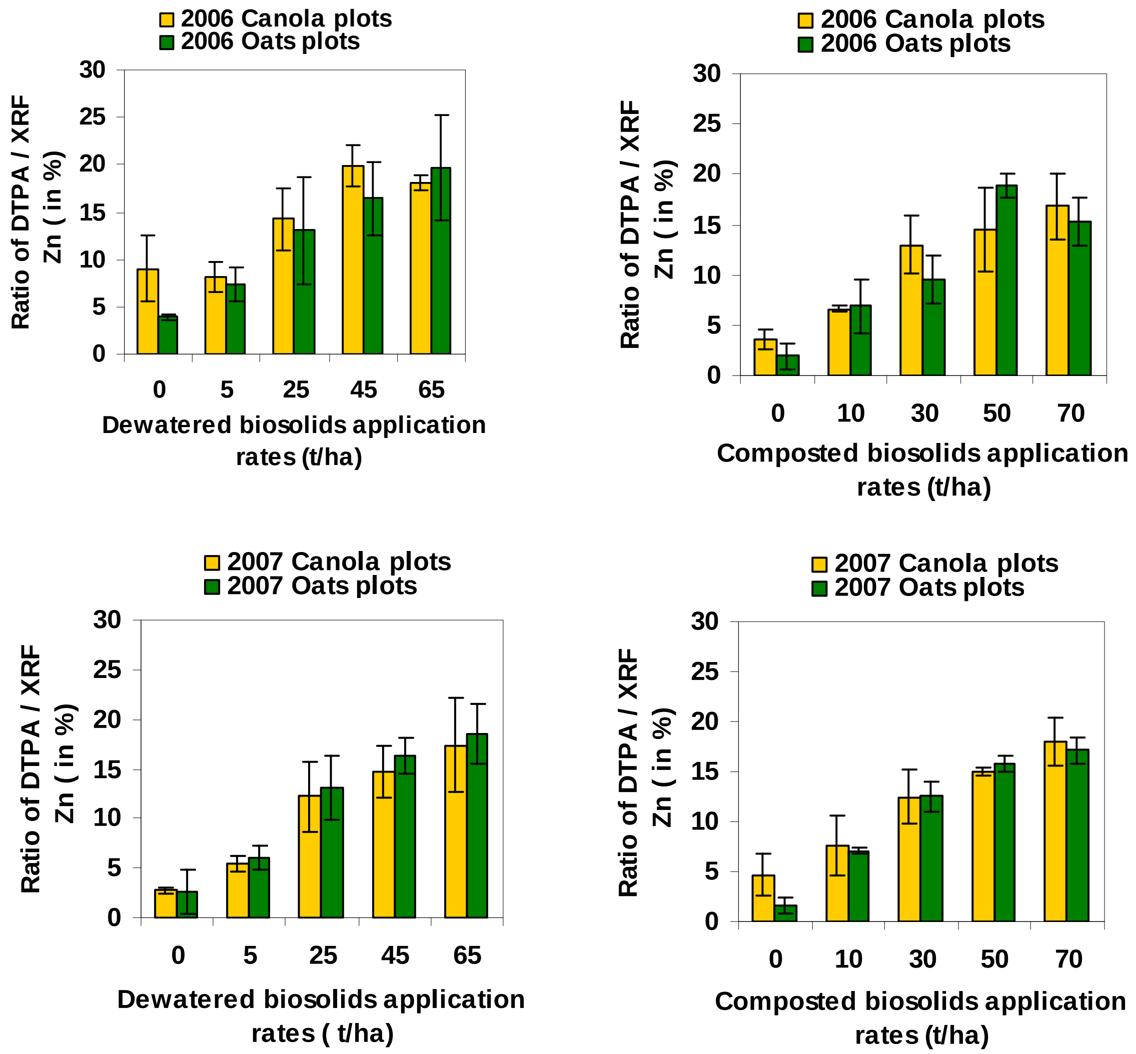


Figure 5.12 The influence of dewatered biosolids applications on the ratio of DTPA to XRF determined soil Zn levels in canola and oats plots in 2006 and 2007 experiments.

The ratio of DTPA to XRF Cu and Zn values in dewatered biosolids treated plots were significantly higher than the corresponding values observed in composted biosolids, (Table 5.1).

The observed increasing trends in the ratio of DTPA to XRF Cu and Zn values were due to two reasons. The first reason would be the dewatered biosolids and composted biosolids had higher concentrations of Cu, Zn and organic matter than the soil background concentrations and thus the upward trend was not unexpected. The second reason would be the nitrification of NH_4-N into NO_3-N releases NO_3-N into the soil which lowers the pH of the soil. Such changes in the soil pH would increase the solubility of Cu and Zn attached in the organic matter matrix resulting in an increase in the ratio of DTPA to XRF determined Cu and Zn concentrations particularly in dewatered biosolids amended soil (Fig. 5.15).

The ratio of DTPA to XRF values for Mn in the 2006 and 2007 canola and oats plots amended with dewatered biosolids showed slight increases, however Mn levels in composted biosolids amended canola and oats plots were relatively constant (Fig.5.13). Higher values for the ratio of DTPA to XRF values for Mn was expected since DTPA extractable Mn concentrations in dewatered biosolids was higher than the levels found in composted biosolids (Table 5.7).

The ratios for Fe in canola and oats plots showed an increasing trend following both biosolids application rates in both years of the experiment; however, the ratios for Fe in dewatered biosolids amended canola and oats plots were considerably higher than the corresponding values observed in composted biosolids amended plots. This was not expected since composted biosolids had slightly higher DTPA extractable Fe than dewatered biosolids, however this difference could be due to higher concentration of organic matter contained in dewatered biosolids than in composted biosolids and the effect it had on lowering the soil pH and thus converting Fe in the soil into a DTPA extractable forms (Fig.5.14).

Similar to Cu and Zn, the ratio values for Fe in oats plots were significantly higher than the values recorded in canola plots. This would be due to differences in the uptake of metals between the two crops. The other reason could be, canola's deeper rooting system may possibly expose the metals for leaching beyond the soil sampling depth leaving lower concentrations of the soluble fractions of the metals on the soil surface.

In general, the ratio of DTPA to XRF determined Cu, Zn and Fe values showed an increasing trend following both biosolids application rates in both years of the experiment. Such increases in the ratio of DTPA to XRF values would be due to the addition of biosolids, and decomposition and mineralization of biosolids organic matter. This process often lowers the soil pH which tends to release heavy metals into the soil thereby increasing the DTPA extractable components.

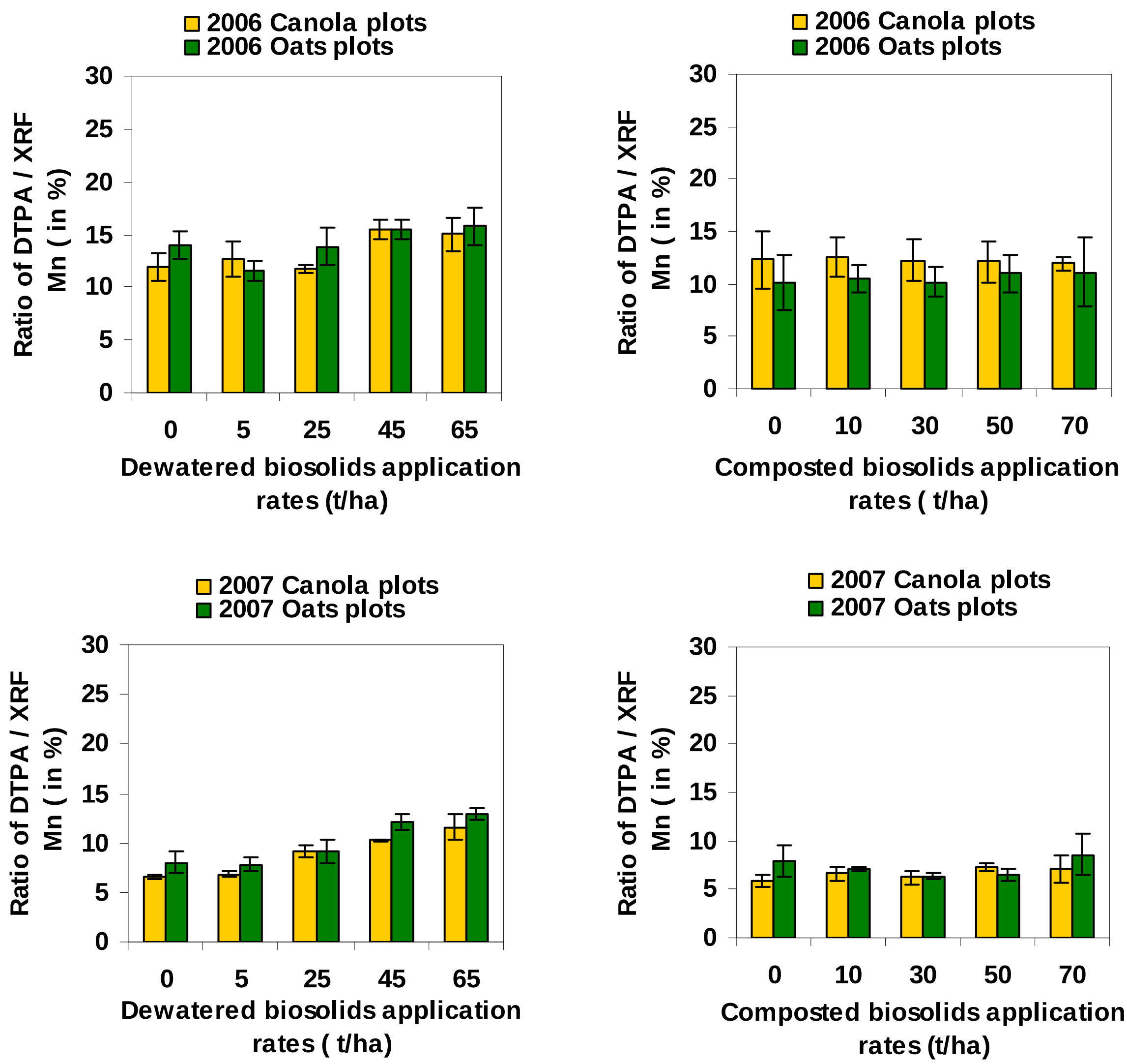


Figure 5.13 The influence of dewatered biosolids applications on the ratio of DTPA to XRF determined soil Mn levels in canola and oats plots in 2006 and 2007 experiments.

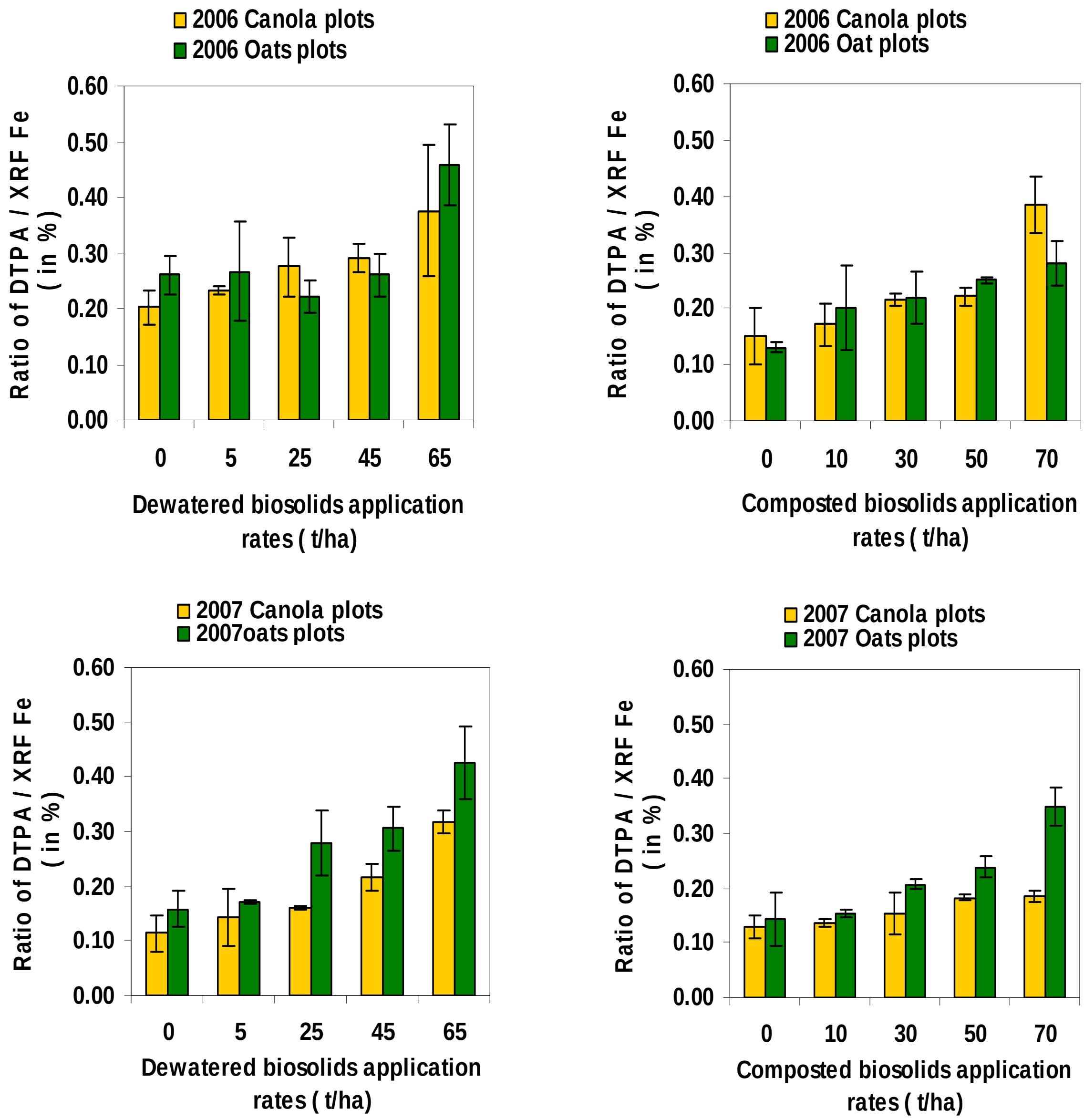


Figure 5.14 The influence of dewatered biosolids applications on the ratio of DTPA to XRF determined soil Fe levels in canola and oats plots, 2006 and 2007 experiments.

5.5. The effect of biosolids applications on soil pH and EC

The change in pH due to biosolids application is of a particular significant since it impacts upon the solubility of heavy metals contained in the biosolids.

Electrical conductivity of soil is an important soil parameter and can be used as an indirect indicator of a number of soil physical and chemical properties. There is a significant positive correlation between electrical conductivity and clay content, cation exchange capacity, soil moisture and organic carbon of soil (Sudduth, *et al*., 2005).

Thus, the Electrical conductivity of soil, biosolids and biosolids amended soil was measured to examine the influence of the two biosolids products on conductivity of the biosolids amended soil and hence if there is any impact on crop productivity.

The application of various biosolids types may or may not alter the soil physical and chemical properties; these include an increase or a decrease in the pH and EC values of the amended soil. The effect of dewatered and composted biosolids applications on soil pH and EC values were investigated under field conditions.

The physicochemical properties of soil, dewatered biosolids and composted biosolids including the pH, EC and CEC are shown in Table 5.14 below. The results indicate that the pH of soil, dewatered biosolids and composted biosolids were relatively similar ranging from 6.4 to 6.7; however, composted biosolids had significantly higher EC values than dewatered biosolids. This is consistent with the higher concentrations the major cations Na^+, K^+, Ca^{++} and Mg^{++} ions in composted biosolids (Table 5.14).

Table 5.14 Results of pH, EC and NH_4Cl extractable exchangeable bases in soil, dewatered biosolids and composted biosolids.

Analytes	Soil	Dewatered biosolids	Composted biosolids
$pH_{w\ (1:5)}$	6.5	6.7	6.4
$EC_{(1:5)}$ (µ S/cm)	67	1350	2704
CEC (meq/kg)	7	24	62
Major cations extracted using 1 M NH_4Cl and analysed by ICP-MS (mg/kg).			
Na	76 ± 3	749 ± 59	1557 ± 50
Mg	264 ± 4	1200 ± 86	3564 ± 17
K	316 ± 11	735 ± 65	4546 ± 79
Ca	697 ± 13	1831 ± 118	2864 ± 519
Al	1.3 ± 0.2	145 ±31	11 ± 9

5.5.1. The influence of dewatered biosolids applications on soil pH and EC

pH

The effect of dewatered biosolids application on soil pH is presented in Fig. 6.15 below.

In the 2006, dewatered biosolids applications had a significant ($p < 0.001$) effect in lowering the soil pH in the oat plots from 6.1 to 5.7 at the highest rate, but , did not do so in the canola plots. In the 2007 experiment, reapplying the same amount of biosolids significantly ($p < 0.001$) affected the pH of soil changing on average from 6.2 to 5.4 and from 6.2 to 5.2 for canola and oats plots, respectively (Fig.5.15). As dewatered biosolids loading rates increased, a decrease in the pH of the amended soil in both years of the experiment was observed which would be due to the decomposition of organic matter in the biosolids releasing H^+ ions in the soil.

Similar findings were also reported by Epstein *et al.* (1976) in which case the application of municipal sewage sludge on crop land decreased soil pH. Antonlin *et al*. (2005) also reported that repeated sludge application at a rate of 15 t/ha reduced the pH and increased the total organic carbon and CEC of the soil.

Several researchers (Helyar 1976; Binkly and Richter 1987; Bolan *et al*. 1991; Kirchmann *et al*. 1996 and Bergkvis *et al*. 2003) suggested that this could be due to the production of NO_3^- ions resulting from mineralization of organic nitrogen and sulfur contained in the biosolids and its accumulation in soil. When NO_3^- ions and basic cations leach from the rooting zone of the soil, there is a net cumulative of hydrogen ions in the soil; one hydrogen ion for every nitrate ion leached, thus the loss of NO_3^- ions accompanied by base cations leaves hydrogen ions in the system thereby decreasing the soil pH. The increase in the concentrations of H^+ due to the mineralization of organic-N is partially shown in the following equation (Brady and Nelson, 2008).

$$\xrightarrow{\hspace{6em}} \text{Mineralization} \xrightarrow{\hspace{6em}}$$

$$R\text{—}NH_2 \underset{+2H_2O}{\overset{-2H_2O}{\rightleftharpoons}} OH^- + R\text{—}OH + NH_4^+ \underset{+O2}{\overset{-O2}{\rightleftharpoons}} 4H^+ + \text{energy} + NO_2^- \underset{-1/2O_2}{\overset{+1/2O_2}{\rightleftharpoons}} \text{energy} + NO_3^-$$

$$\xleftarrow{\hspace{6em}} \text{Immobilization} \xleftarrow{\hspace{6em}}$$

Similarly, Alves *et al*. (2006) in their field experiment also observed a decrease in soil pH as the application of biosolids increased, and suggested that the acidic biosolids used in the experiment and the decomposition of soil organic matter by microorganisms could produce organic acids, together with carbon dioxide and water, resulting in the formation of carbonic acid.

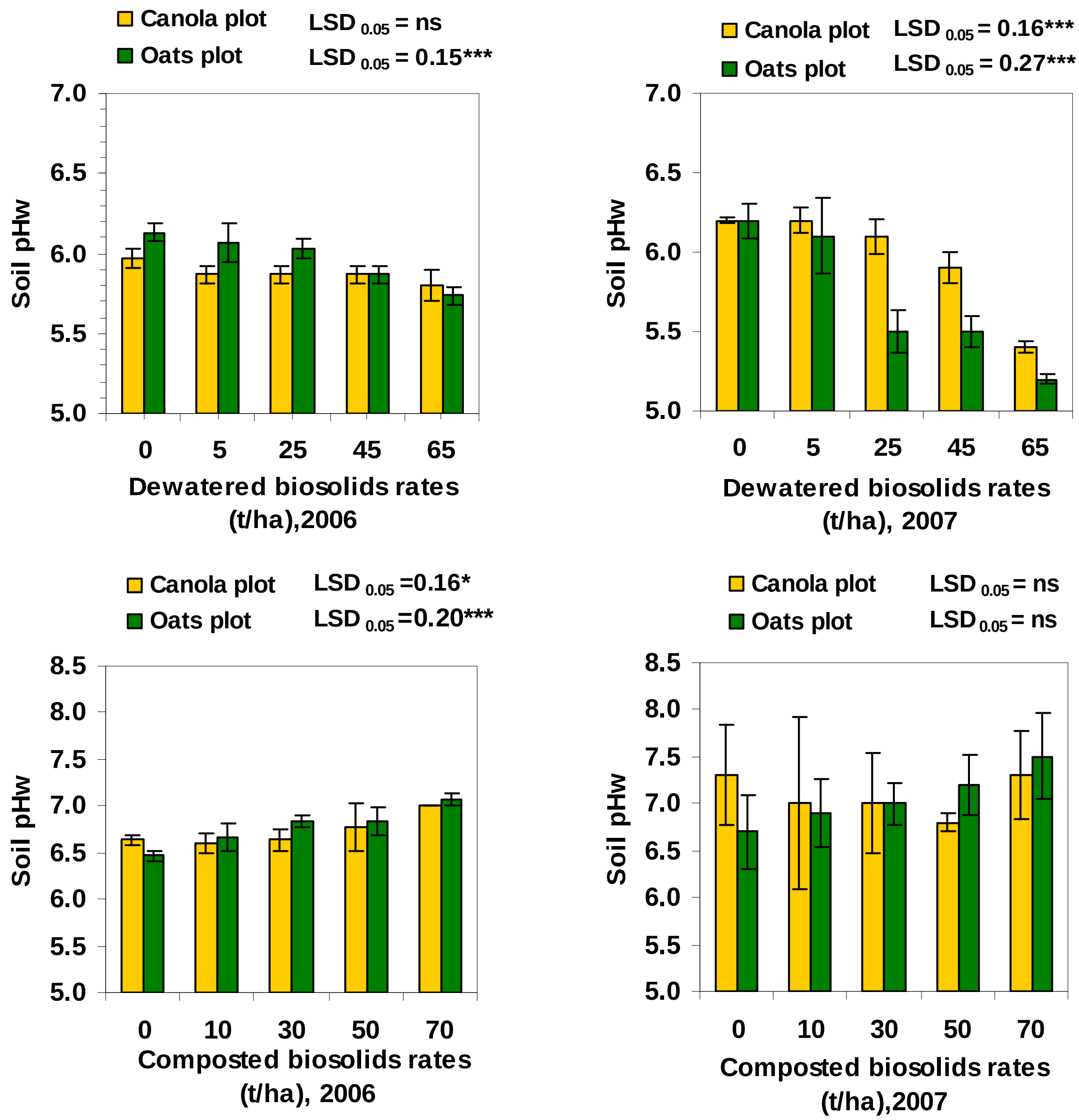


Figure 5.15 The effect of dewatered and composted biosolids application rates on soil pHw in 2006 and 2007 field experiments. The error bars represent standard deviations of triplicate measurements. The LSD $_{0.05}$ values refers to the least significant difference for the means at 5% probability, whereas*, *** and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.001$ and not significant respectively ($n = 3$).

Impellitteri *et al.* (2003) also found that soil pH_w and percent organic matter explained 80% of the variability in plant-available copper (as estimated by 0.01 M HCl extraction) and pH alone explained 80% of the variability of plant available zinc (as estimated by 0.01 M $CaCl_2$ extraction) in a set of 40 soils from the United States, Canada, the United Kingdom, the Netherlands, and Chile with diverse characteristics.

The decrease in pH following dewatered biosolids applications may have impacted on heavy metals solubility in biosolids amended soil which agree with the findings of Efroymson *et al.* (2001) where pH_w was a statistically significant variable for accumulation of cadmium and zinc by plants in field soils, and the multiple regression incorporating soil concentration of the element and pH_w predicted the plant concentration of Cu and Pb in a validation data set better than models without pH_w.

Therefore, in the long term, nitrification of the NH_4-N into NO_3-N and the mineralization of S from the biosolids can have more impact on the pH of the amended soil than the pH of the biosolids itself since in our study the pH values of the soil and the biosolids were almost similar at the beginning of the study.

The effect of dewatered biosolids in decreasing the soil pH at the higher application rates was slightly higher for soil samples taken from oat plots than in the canola plots during the second year of the experiment.

Over all, the change in soil pH did not affect the yields of crop, since both crops can grow and give good yields within these pH ranges. The minimum pH_w for canola is thought to be 5.5 below which the crop is likely to be stunted and unable to reach its yield potential, nevertheless, canola can grow on calcareous soils, up to $pH_w = 8.5$ (Potter *et al.*,1999).

Maximum oats yields are usually achieved within 5.3-5.7 pH_w ranges (Adam and Amam, 1979; Doll, 1964), oats have been shown to withstand acid soils with a pH_w of 4.5 (Stoskopf, 1985)

EC

The electrical conductivity data, despite some variability shows that the application of dewatered biosolids in 2006 significantly increased the electrical conductivity of the amended soil on average changing from 159 in the control plot to 216 $\mu S\ cm^{-1}$ in the 65 t/ha in the canola plots but this effect was not observed in the oat plots.

A comparison of the control and the highest dewatered biosolids (65 t/ha) treated plots after reapplying dewatered biosolids in 2007 shows that on average there was an increase in the electrical conductivity of the soil from 62 to 150 $\mu S\ cm^{-1}$ in the canola plots and from 71 to 126 in oats plots respectively (Table 5.15). Even though the EC values recorded in 2007 dewatered biosolids treated plots showed an increasing trend, they were significantly less than the 2006 recordings. This could possibly be due to the production of NO_3^- and SO_4^{2-} ions resulting from mineralization of organic nitrogen and sulphur contained in the biosolids and accompanied by the leaching of major cations (Na^+, K^+, Ca^{++} and Mg^{++}) down the soil profile. In addition, the experimental plots were irrigated (tap water) using sprinkler watering system and this may have enhanced the down ward leaching of soluble salts through time.

Table 5.15 Effect of biosolids application rates on EC of amended soil in 2006 and 2007 field experiments.

Dewatered biosolids rates (t/ha)	Canola Ec μScm^{-1}	Oats Ec μScm^{-1}	Composted biosolids rates (t/ha)	Canola Ec μScm^{-1}	Oats Ec μScm^{-1}
Biosolids amended soil samples taken from 2006 experiments					
0	159 ±51	175±31	0	192±17	215±35
5	119±35	138±45	10	173±78	239±41
25	147±35	148±39	30	188±36	161±62
45	226±58	182±38	50	234±22	211±79
65	215±9	144±25	70	258±57	220±57
F	177±26	158±22	F	232±43	197±97
$LSD_{0.05}$	71*	ns	$LSD_{0.05}$	ns	ns
Biosolids amended soil samples taken from 2007 experiments					
0	62±1	71±13	0	62±11	72±3
5	72±10	71±11	10	85±7	78±10
25	124±13	127±13	30	110±26	72±7
45	123±16	110±14	50	118±24	90±23
65	151±19	126±19	70	139±19	112±1
F	81±12	77±15	F	91±21	93±5
$LSD_{0.05}$	26***	25.3***	$LSD_{0.05}$	40*	24*

The superscripts *,**,*** and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively, values indicate means of triplicate measurements (n = 3).

5.5.2. The influence of composted biosolids applications on soil pH and EC of the amended soil

pH

In the 2006 experiment, when the control and the highest composted biosolids applied plots (70 t/ha) were compared, the effect of composted biosolids application rates on soil pH_w was significant, on average increasing from 6.7 to 7.0 in the canola plots and from 6.5 to 7.1 in the oats plots respectively. As composted biosolids application rates increased, soil pH_w also showed an increasing trend in both canola and oats plots. In contrast to dewatered biosolids, increases in soil pH_w observed in 2006 composted biosolids amended plots were attributed to the high concentration of exchangeable base cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) and the relatively higher EC values of the composted biosolids. However, reapplying composted biosolids in 2007 had no significant effect on the pH_w of the soil. Indeed, repeated application of composted biosolids in the second year of the experiment could have increased the level of base cations in the soil, however the simultaneous build up of H^+ which resulted from the mineralization of organic nitrogen contained in the composted biosolids would probably off-set the increase in soil pH (Fig.5.15). Several researchers (Summers, 1977; Kim *et al.*, 2007) also reported similar findings where the addition of composted biosolids increased the pH of soil. Most researchers (Sims and Kline 1991; Tsadilas *et al*. 1995; Chlopecka 1996b; Su and Wong 2003) reported increases in soil pH following the applications of Municipal sewage sludge, composted biosolids and coal fly ash stabilized biosolids on a variety of soils under green house and field conditions. Such pH changes after increasing ash and composted biosolids amendments may have caused by the precipitation of soluble cations in the sludge mixture raising the soil pH.

In this study, the two biosolids types impacted upon the soil pH differently where dewatered biosolids decreased the soil pH, whereas an increase in pH followed the application of composted biosolids applications. Similar changes in soil pH have been attributed to the calcium carbonate content of sludge and production of acid during sludge decomposition (Sommers, 1977).

EC

In the 2006 composted biosolids treated canola and oats plots, EC values were not significantly different from the control plots. However, a significant increasing trend in EC values in the 2007 composted biosolids treated canola and oats plots were noted, as expected because of the high EC values of the composted biosolids. Consistent with this finding, Martinez *et al*., (2002) also reported increases in EC of soil following urban organic wastes applications.

In contrast, both biosolids types had similar effect on EC, slightly increasing the EC values of the amended soil (Table 5.15). Nevertheless, neither crop would have been affected by these increases since canola yields can only be affected when the EC of the saturation extract is more than 40×10^{4} μScm^{-1} (Potter *et al*., 1999). Oats are slightly salt tolerant (Bresler *et al*., 1982), and under non-saline conditions oats are moderately tolerant to sodium (Pearson, 1960).

5.6. Effect of cropping sequence on DTPA extractable heavy metals

Based on the DTPA extractable metals data presented in appendix J Table J-1 to Table J-4, section 5.6 of the thesis describes the influence of two years crop rotation on Cu, Zn and Fe residues left in the soil after successive biosolids application. In order to examine the effect of crop rotation on metals residue left in the soil, the maximum dewatered biosolids and composted biosolids treated plots were chosen (which would presumably magnify the effect) and the corresponding metal residueals were compared over the two cropping seasons

The study showed that crop rotation had significant effect on DTPA extractable heavy metals residues accumulated at the 0-10 cm soil depth after two years application of dewatered biosolids and composted biosolids. For the canola-oats cropping sequence in dewatered biosolids amended plots, on average 5, 10 and 65 mg/kg of DTPA extractable Cu, Zn and Fe residues were observed, whereas for the oats-canola cropping sequence, 3.2, 5 and 9 mg/kg of Cu, Zn and Fe were found in the 0-10 cm depth of the soil.

Table 5.16 The changes in DTPA extractable Cu, Zn and Fe residues due to canola and oats cropping sequence in biosolids amended soil in 2006 and 2007 field experiments, (expressed in mg/kg)

Cropping sequence in dewatered biosolids treated canola and oats plots							
Analytes	Yr 1 canola	Yr 2 oats	Yr 2-Yr1	Analytes	Yr 1 oats	Yr 2 canola	Yr 2-Yr1
Cu	3.3	8.2	5	Cu	3.1	6.3	3.2
Zn	6.0	15.5	10	Zn	7.6	12.8	5.2
Fe	37	103	65	Fe	78	87	9
Cropping sequence in composted biosolids treated canola and oats plots							
Analytes	Yr 1 canola	Yr 2 oats	Yr 2-Yr1	Analytes	Yr 1 oats	Yr 2 canola	Yr 2-Yr1
Cu	1.2	2.0	0.8	Cu	2.5	2.2	-0.3
Zn	8.7	23.2	14.5	Zn	18	18	0
Fe	77	103	26	Fe	62	30	-32

Dewatered and composted biosolids amended soil samples were taken after each year crop harvest and the plant available fractions of Cu, Zn and Fe were extracted using DTPA solution and the concentrations were determined by ICP-MS. The unamended control and the highest dewatered (65 t/ha) and composted (70 t/ha) biosolids treated plots were compared based on metals residues in soil after the end of 2006 and 2007 crop harvest.

Likewise, in composted biosolids treated plots canola-oats cropping sequence, on average 0.84 Cu, 14.4 Zn and 26 µg/g Fe residues were recorded; however, in the oats-canola rotation 0.34 µg/g Cu, - 0.24 Zn µg/g and - 31 Fe µg/g reductions at the end of the 2007 experiments were noted. The negative values indicate Zn and Fe levels recorded at the end of the 2006 experiment were greater than the levels recorded at the end of the 2007 trial (Table 5.16).

It could be possible that the higher concentrations of DTPA extractable metals observed in the canola-oat rotation may be due to the increased up take of metals by canola compared to oats (Table 5.24 and 5.25) in the first year of the experiment reducing the DTPA extractable levels of the canola plots. This might have led to greater concentrations of metals in the oats plots in the second year of the experiment, resulting in higher DTPA extractable concentrations of metals in the canola-oats rotation compared to the oats-canola rotation.

Likewise, the pH of oats plots amended with dewatered biosolids was lower compared to canola; this could also partly explain the larger DTPA residue in the second year after oats (Figure 5.15). Moreover, the performance of the oat crop in 2007 was reduced due to the incidence of crop pest infestation and as the result of this; the oats would not have been able to take up as much as usual.

In addition, the decomposition of biosolids organic matter which releases heavy metals into the soil in the first year of the experiment may take some time, thus at the end of the canola-oats rotation a substantial amount of DTPA extractable Zn and Fe residue may accumulate in the amended soil, because the oat crops are not as efficient in taking up the residual Zn and Fe as the canola. On the other hand, in the oats-canola rotation the canola crop would probably get sufficient time to take up most of the available Zn and Fe residue at the end of the two years experiment leaving a lesser amount of Cu and Fe residue in the amended soil. This would be consistent with the significantly higher uptake of Zn by canola than the oat crops.

Thus, in the oats-canola cropping sequence, significantly lower concentrations of available Zn and Fe were observed than in the canola-oats sequence in both dewatered and composted biosolids treated plots which suggests that crop rotation had a considerable effect on the levels of Zn and Fe residues left in the amended soil after two years of biosolids applications (Table 5.16).

5.7. Plant uptake of heavy metals in biosolids amended soil

The bioavailability of heavy metals such as Cu and Zn would be an important factor from nutrient release stand point to enhance crop growth and from land contamination perspectives where repeated application of biosolids may elevate the concentration of these elements to levels that possibly contaminate the receiving soil.

Soil pH, organic matter and clay content and interaction of elements in the biosolids amended soil determine the solubility of heavy metals and thus uptake and accumulation by plants which could possibly become phytotoxic under excessive conditions.

Heavy metals accumulation in plants differs with plant species, cultivars and plant parts. In most cases, heavy metals are more concentrated in the leaves than in the grain.

Thus, to investigate the concentrations of various heavy metals in the leaves of canola and oats amended with dewatered and composted biosolids, plant samples were taken at 4-5 leaf stages for canola and at 5-6 tillering stages for oats and were analysed to examine the effect of biosolids application rates on heavy metals concentration in the leaves and their possible phytotoxicity or deficiency.

5.7.1. Analytical quality assurance

For HNO_3/H_2O_2 extractable pseudo total metal concentrations in plant leaves, a standard reference material SRM 1573a (Tomato leaves) was analysed in duplicate for every 18 samples and the percent recovery was determined.

To check the reproducibility of the extraction technique, five replicates of plant samples were separately extracted and analysed. The coefficient of variation was calculated.

The recovery values for the standard reference material for SRM1573a tomato leaves were slightly lower than the certified values. This was not unexpected, since the values obtained for SRM 1573a reference material had been obtained using perchloric, nitric, hydrochloric and hydrogen fluoride acid digestion, whereas in this work, the reference material was digested using HNO_3/H_2O_2. Hence the amounts determined by this method will be slightly underestimating plant uptake. In general, the recoveries of the measured values for all the heavy metals were high (Table 5.17).

Table 5.17 The measured and certified values of standard reference material (SRM 1573a tomato leaves) analysed using ICP-MS for the purpose of validating the analytical data for canola and oats leaves analysis (mg/kg on dry weight basis).

Analytes	Measured	Certified	Recovery (%)
Cu	4.1 ± 0.1	4.70	87
Zn	45 ± 6	30.90	120
Mn	201 ± 1	246	82
Fe	372 ± 6	368	101
Co	0.9 ± 0.6	0.57	88
Ni	1.4 ± 0.1	1.59	83

The measured values indicate mean ± standard deviations of duplicate measurements

5.7.2. Heavy metals uptake by canola

Results of plant tissue analyses in the 2006 experiment showed that when the control plot was compared with the highest dewatered biosolids application rates (65 t/ha), the uptake of Cu, Zn and Mn by canola crop increased significantly ($p < 0.05$) from 10.3 to 15.8 µg/g dm for Cu, from 63.6 to 115.4 µg/g dm for Zn and from 34.6 to 54 µg/g dm for Mn, respectively.

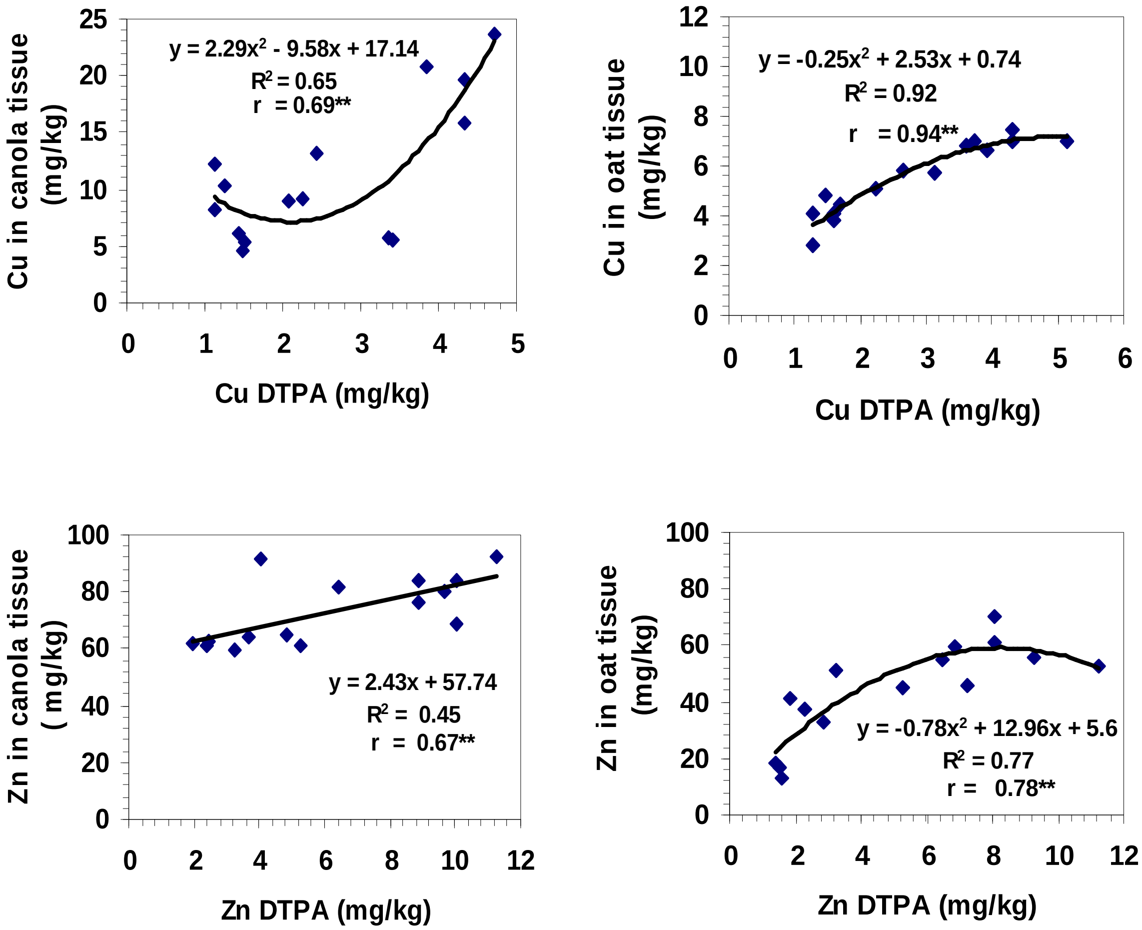


Figure 5.16 Relationships between DTPA extractable Cu and Zn in soil and their respective concentrations in canola and oat tissue in the 2006 dewatered biosolids amended plots in field experiment. Values are expressed on dry matter basis. The superscripts ** refers to significant Pearson's correlation at $p < 0.01$ level.

The concentrations of Cu and Zn in canola tissue were positively correlated ($r = 0.69^{**}$ and $r = 0.67^{**}$) with their respective DTPA extractable soil concentrations, Cu showing a quadratic trend as biosolids application rates increases whereas Zn showed a linear trend (Fig. 5.16).

In contrast, in the 2007 experiment Cu and Zn in canola tissue were not significantly different from the control plots although a significant increase in tissue Mn was observed changing from 40 to 118 mg/kg at the highest (65 t/ha) biosolids rates ($p < 0.05$).

Similarly, a relatively linear increase in the uptake of Cu and Zn by canola crop following increased composted biosolids application was also observed. Despite a significant correlation ($r = 0.61^{*}$) between Zn in canola tissue and DTPA extractable Zn in soil, the correlation between Cu in canola tissue and DTPA extractable Cu levels was not significant (Fig. 5.17 and Appendix F).

Effect of total XRF metals and soil pH on metals uptake by canola

The relationship between soil total metals (XRF total), soil pH and metal concentrations in plant tissues were examined using a multiple regression analysis.

Before multiple regression was conducted, total Cu and Zn concentrations recorded in the unamended control plots of soil and plants were subtracted from Cu and Zn values obtained from the various dewatered biosolids and composted biosolids treated soil and plants and the intercept was set to zero. This was done to distinguish only the influence of biosolids applications and hence concentrations of Cu and Zn in canola leaf were regressed against soil total Cu and Zn concentrations and soil pH. The results are summarized in Table (5.18).

Results of the analysis showed that although application of dewatered biosolids lowered soil pH and increased total Cu and Zn residue in the amended soil, the change in soil pH and total Cu and Zn levels had no significant effect on the concentrations of Cu and Zn in canola leaf in both years of the experiment Table 5. In composted biosolids treated canola plots, total soil Zn levels and pH were not related to the concentration of Zn observed in canola tissue in both years of the experiment, but soil pH in both years of the experiments had a positive impact on the concentration Cu in canola tissue . In the 2007 experiment, Cu residue in soil was negatively related to the concentrations of Cu in canola tissue (Table 5.18).

Table 5.18 The impact of XRF total metals and soil pH on HNO_3/H_2O_2 extractable total Cu and Zn concentrations in canola leaves in 2006 and 2007 field experiments, multiple regression analysis.

Analytes	Dewatered biosolids amended canola plot				Composted biosolids amended canola plot			
	Regression Coefficients		Adjusted R^2	P- value	Regression Coefficients		Adjusted R^2	P- value
	XRF	pH			XRF	pH		
Cu 2006	0.6^{ns}	-0.3^{ns}	0.20	0.33	0.02^{ns}	0.32^{*}	0.65	0.007
2007	0.04^{ns}	0.04^{ns}	0.06	0.29	-0.05^{*}	0.21^{**}	0.56	0.01
Zn 2006	0.50^{ns}	0.95^{ns}	0.50	0.006	0.97^{ns}	0.90^{ns}	0.46	0.011
2007	0.81^{ns}	17^{ns}	0.51	0.027	0.034^{ns}	3.8^{ns}	0.44	0.047

The regression coefficients estimated for the independent variables XRF total Cu and Zn and soil pH were tested whether the values were statistically different from zero or not, thus the superscripts *, **, *** and ns refer to significant levels (t-test) for the regression coefficients at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively. The superscript "b" indicates data not analysed because in the 2007 plot experiment, the oat crop were infested with stem/leaf rust which negatively impacted the over all performance of the oat crop.
The p- values stand for the level of significance for the regression line (R^2) ANOVA (F-test).

5.7.3. Heavy metals uptake by oats

Although the concentrations Cu, Zn and Mn in oat tissue was lower than those found in canola tissue, in the 2006 experiment, there were significant increases ($p < 0.001$) in Cu, Zn and Mn in oat tissue changing from 3.6 to 7 mg/kg dm for Cu, from 16 to 61.4 mg/kg dm for Zn and from 75 to 101 mg/kg dm for Mn respectively. The highest tissue Mn (101 mg/kg dm) was recorded at the 5 t/ha biosolids application rates. A significant positive correlation ($r = 0.94^{**}$ and $r = 0.78^{**}$) between Cu and Zn in oat tissue and their respective DTPA extractable concentrations in soil were also noted and showed a plateau type response (Fig.5.16).

Likewise, in 2006, Cu and Zn levels in oats tissue were significantly different from the control plots and changed from 3.8 to 5.5 mg/kg dm for Cu and from 18 to 46 mg/kg dm for Zn respectively. The correlation coefficients between Cu and Zn in oats tissue and their respective DTPA extractable concentrations were $r = 0.73$ and $r = 0.70$ respectively and showed a plateau type response following composted biosolids application rates (Fig. 5.17 and Appendix F); however, in 2007, the responses of Cu and Zn to increased composted biosolids rates were not significantly different from the control plot.

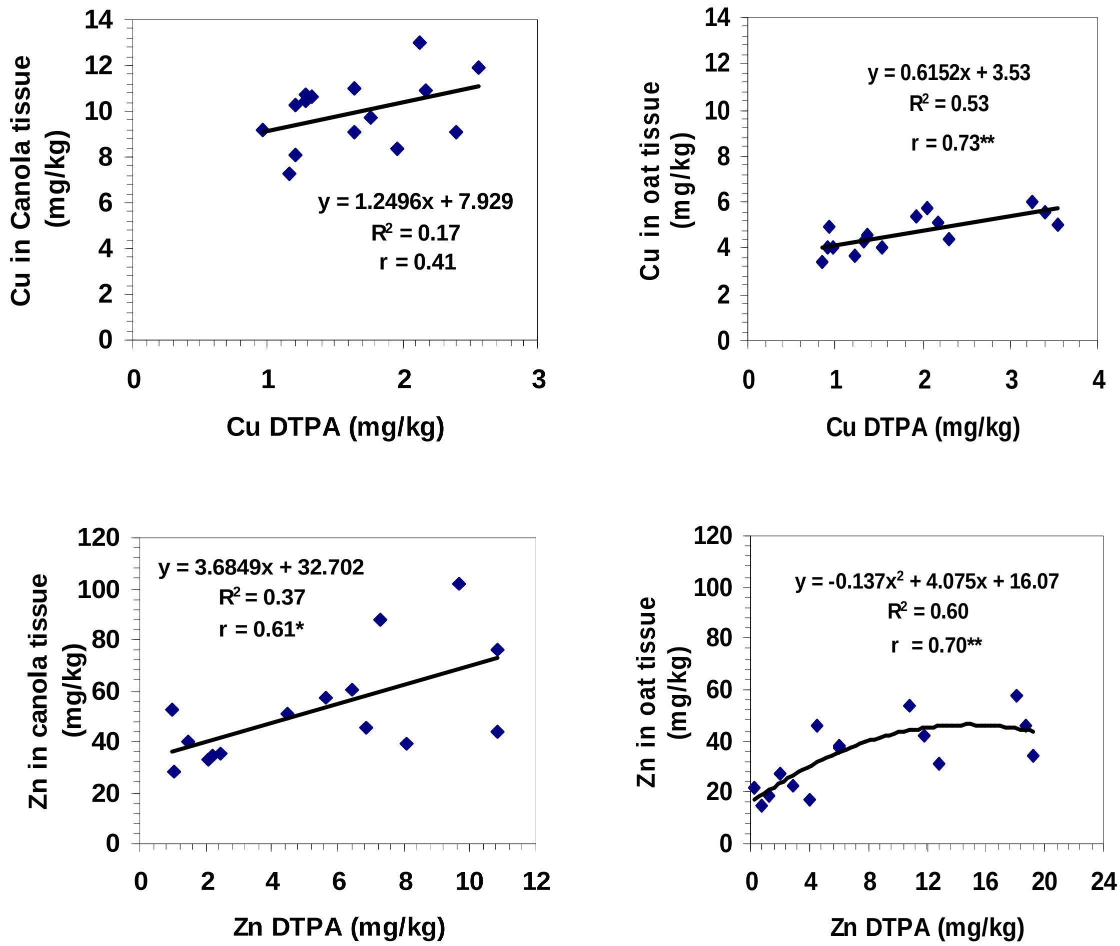


Figure 5.17 Relationships between DTPA extractable Cu and Zn in soil and their respective concentrations in canola and oat tissue in 2006 composted biosolids amended plots. Values are expressed on dry matter basis. The superscripts *, ** and ns refers to significant Pearson's correlation coefficient at $p < 0.05$, $p < 0.01$ and not significant respectively.

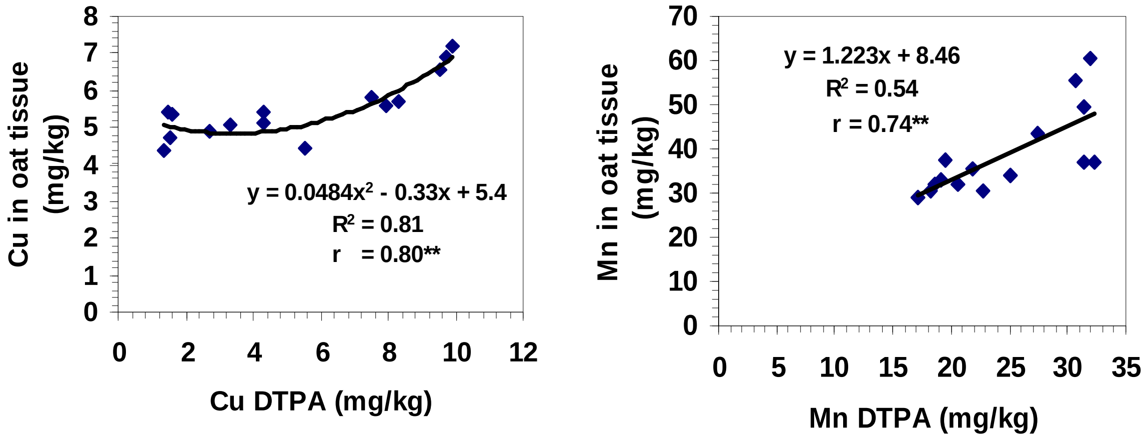


Figure 5.18 Relationships between DTPA extracted Cu and Mn in soil and their respective concentrations in oat tissue in 2007 dewatered biosolids amended plots in field experiment. Values are expressed on dry matter basis.

Effect of total XRF metals and soil pH on metals uptake by oat

In the 2006 dewatered biosolids treated oat plots, the XRF total Cu and Zn were positively related to the corresponding concentrations of Cu and Zn in oat tissue. In addition, soil pH positively impacted on the concentrations of Zn in oat tissue. Similarly, in the 2006 composted biosolids treated oat plots, significant positive regression coefficients were observed for XRF total Cu and Zn values indicating that metal loading had positive impact on Cu and Zn concentrations in oat tissue. Soil pH did not affect the level of Cu in oat tissue, but had a positive impact on the concentration of Zn in oat tissue (Table 5.19).

Table 5.19 The impact of XRF total metals and soil pH on HNO_3/H_2O_2 extractable total Cu and Zn levels in oat leaves in 2006 and 2007 field experiments, multiple regression analysis.

Analytes	Dewatered biosolids amended oat plot				Composted biosolids amended oat plot			
	Regression Coefficients		Adjusted R^2	P- value	Regression Coefficients		Adjusted R^2	P- value
	XRF	pH			XRF	pH		
Cu 2006	0.21^{**}	0.15^{ns}	0.83	< 0.001	0.045*	0.02ns	0.79	0.001
2007	na^{b}	na	na	na	na^{b}	na	na	na
Zn 2006	1.3^{***}	3.4^{***}	0.86	< 0.001	0.25*	1.7*	76	0.001
2007	na	na	na	na	na^{b}	na	na	na

The regression coefficients estimated for the independent variables XRF total Cu and Zn and soil pH were tested whether the values were statistically different from zero or not, thus the superscripts *, **, *** and ns refer to significant levels (t-test) for the regression coefficients at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively. The "na" indicates data not analysed because in the 2007 plot experiment, the oat crop were infested with stem/leaf rust which negatively impacted the over all performance of the oat crop. The p- values stand for the level of significance for the regression line ($R^{2)}$ ANOVA (F-test).

Table 5.20 Changes in the level of heavy metals and Mn in canola and oats leaves due to dewatered and composted biosolids applications in 2006 field experiment (mg/kg)

Plant samples taken from dewatered biosolids treated canola and oats plots, 2006						
Dewatered biosolids rates(t/ha)	Concentrations of heavy metals in canola leaf expressed in mg/kg, 2006			Concentrations of heavy metals in oats leaf expressed in mg/kg, 2006		
	Cu	Zn	Mn	Cu	Zn	Mn
0	10 ± 2	64±1	35±8	4±1	16±3	75±4
5	10 ± 1	61±1	48 ±8	4.4±0.4	37±4	97±6
25	10 ± 2	78±15	52±10	5.5±0.4	48±3	67±10
45	11 ± 4	82±12	45 ±5	6.9±0.1	57±2	56±7
65	16 ± 4	115±4	54±6	7.0±0.4	61±9	77 ±4
$LSD_{0.05}$	8.3*	16.4*	12.4*	0.8***	8.9***	12**
Measured	3.4±1.6	23.5±0.4	197±7	5.7±0.9	21.5±0.9	182 ±1
Certified	4.7	30.9	246	4.7	30.9	246
Recovery	72 (%)	76 (%)	80	121 (%)	70 (%)	74 (%)
Plant samples taken from composted biosolids treated canola and oats plots, 2006						
Composted biosolids rates (t/ha)	Concentrations of heavy metals in canola leaf expressed in mg/kg, 2006			Concentrations of heavy metals in oats leaf expressed in mg/kg, 2006		
	Cu	Zn	Mn	Cu	Zn	Mn
0	8 ± 1	46 ± 9	34 ± 3	3.8 ± 0.3	18.3 ± 0.3	57 ± 4
10	10.5 ± 0.2	34 ± 1	46 ± 11	4.3 ± 0.6	22 ± 5	41 ± 3
30	10 ± 1	51 ± 6	39 ± 3	4.6 ± 0.7	41 ± 5	43 ± 11
50	9.1 ± 0.7	83 ± 21	36 ± 4	5.1 ± 0.7	42 ± 11	31 ± 5
70	12 ± 1	53 ± 20	39 ± 3	5.5 ± 0.5	46 ± 12	28 ± 4
$LSD_{0.05}$	1.6**	26*	ns	1.0*	14**	11***
Measured	3.1 ± 0.2	25 ± 8	197 ± 21	3.2 ±0.7	19±6	180±1
Certified	4.7	30.9	246	4.7	30.9	246
Recovery	65 (%)	81 (%)	80 (%)	68 (%)	62 (%)	73 (%)

Values indicate means of triplicate measurements ,the superscripts *, **,*** and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively, whereas the recoveries for the measured and certified values stand for NIST standard reference material SRM 1573 a (tomato leaves). The LSD 0.05 indicates the least significant difference (t-test at $p <0.05$ levels) between the mean values for Cu, Zn and Mn respectively.

Table 5.21 Changes in the concentrations of heavy metals and Mn in canola and oats leaves due to dewatered and composted biosolids applications in 2007 field experiment (mg/kg)

Plant samples taken from dewatered biosolids treated canola and oats plots, 2007						
Dewatered biosolids rates(t/ha)	Concentrations of heavy metals in canola leaf expressed in mg/kg, 2007			Concentrations of heavy metals in oats leaf expressed in mg/kg, 2007		
	Cu	Zn	Mn	Cu	Zn	Mn
0	7.8 ± 1.0	103 ± 70	31 ± 4	5.2 ± 0.4	48 ± 5	32 ± 3
5	6.9 ± 0.8	95 ±5 1	32 ± 4	5.0 ± 0.5	28 ± 8	32 ± 1
25	6.5 ± 0.1	177 ± 137	37 ± 4	5.0 ± 0.4	56 ± 14	34 ± 4
45	6.7 ± 0.3	71 ± 3	60 ± 15	6.0 ± 0.1	44 ± 6	39 ± 4
65	7.1 ± 0.6	167 ± 54	118 ± 31	7.0 ± 0.3	53 ± 12	55 ± 5
$LSD_{0.05}$	ns	ns	28.9*	0.7*	ns	6.8***
Measured	3.33 ± 0.04	26.00 ±0.01	197 ± 1	3.1 ± 0.2	37±1	177 ± 1
Certified	4.7	30.9	246	4.7	30.9	246
Recovery	69 (%)	84 (%)	80 (%)	65 (%)	119 (%)	72 (%)
Plant samples taken from composted biosolids treated canola and oats plots, 2007						
Composted biosolids rates (t/ha)	Concentrations of heavy metals in canola leaf expressed in mg/kg, 2007			Concentrations of heavy metals in oats leaf expressed in mg/kg, 2007		
	Cu	Zn	Mn	Cu	Zn	Mn
0	6.4 ± 0.2	63 ± 18	33 ± 5	6.4 ± 1.1	nd	31 ± 3
10	7.4 ± 0.5	97.5 ± 0.1	33.0 ± 0.1	5.6 ± 0.1	nd	30 ± 4
30	6.6 ± 0.1	61 ± 13	43 ± 8	5.17 ± 0.03	nd	24 ± 1
50	5.8 ± 0.3	60 ± 5	44 ± 9	7.5 ± 1.9	nd	24 ± 2
70	5.6 ± 0.6	103 ± 24	33 ± 4	5.6 ± 0.5	nd	23 ± 4
$LSD_{0.05}$	0.7**	27*	ns	ns	ns	5.3*
Measured	4.08 ± 0.11	45 ± 6	201 ± 0.8	4.1 ± 0.9	-	168 ± 8
Certified	4.7	30.9	246	4.7	-	246
Recovery	87 (%)	120 (%)	82 (%)	86 (%)	-	68 (%)

Values indicate means of triplicate measurements ,the superscripts *, **,*** and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively, whereas the recoveries for the measured and certified values stand for NIST standard reference material SRM 1573 a (tomato leaves). The LSD indicates the least significant difference (t-test at $p <0.05$ levels) between the mean values for Cu, Zn and Mn.

Discussion

Several researchers (Hua *et al*. 2007; Samaras and Tsadilas 1997; Tsadilas *et al*. 1995) have reported a positive correlation between the concentration of heavy metals extracted by DTPA method and the respective concentrations in the plants.

At low pH, heavy metals are more soluble (in the soil solution) and mobile. This increases their potential uptake by plants and greater possibility of movement through the soil profile (Epstein, 2003). McBride (1995) contended that as the organic matter decomposed, trace elements will be released into more soluble forms and result in increased uptake of metals from biosolids.

A number of researchers also suggested that the uptake of metals by various crops was not linear with heavy metals or sludge/biosolids' application rate, but rather approached a maximum and then levelled or decreased, showing a plateau type response (Chaney and Ryan, 1992; Logan and Chaney, 1983; Corey *et al.*, 1987).

The 2006 experiment showed that the uptake of Cu and Zn by canola was significantly higher than the oat crops in both dewatered biosolids and composted biosolids experiments, concentrations of Cu and Zn showed increasing trends.

In contrast, Cu in oat tissue increased at decreasing rates, whereas Zn exhibited a plateau type response where Zn levels in oats tissue increased and declined as biosolids loading rates increased (Fig. 5.16 and 5.17).

Thus, the response of oats in terms of metal uptake from biosolids amended soil showed a plateau type response and was different from the canola crop.

In the 2007 experiment, Cu and Mn in oats tissue were significantly different from the control plots ($p < 0.01$), but concentration of zinc was not different from the control plots ($p > 0.05$).

The quantity of dewatered biosolids added during the two years periods was expected to be 12 fold (NLBAR x 6 x 2 applications) times the recommended rate; however concentrations of Cu and Zn in canola shoot were very low compared with the unamended control plants demonstrating that there were negligible effects of biosolids applications at normal agronomic application rates on metal bioavailability at least in the short to medium.

Wen *et al*. (2002a) conducted series of field experiments to evaluate the effects of dewatered digested, digested-irradiated and composted sludge, and composted livestock manure applied to a loamy sand soil (pH 6.6) on plant availability of Zn. The biosolids were applied up to a maximum rate of 40 t DS /ha/yr during two growing seasons and the crops grown were lettuce and snap bean and an ornamental plant. Composted biosolids significantly decreased the availability and crop uptake of Zn compared to the uncomposted sludges, moreover Zinc applied in composted sludge did not increase the crop Zn concentration.

A positive correlation between Cu and Mn in oat tissue and their respective DTPA extractable concentrations in soil ($r = 80^{**}$ and $r = 0.74^{**}$) were also observed which showed a slightly increasing trend as their respective extractable concentrations in soil increased (Fig.5. 18).

Generally, DTPA extractable concentrations of Cu and Zn in soil and their respective concentrations in canola and oats leaf in the 2006 experiment were positively correlated indicating that the DTPA extraction can be used as a good predictor of plant available heavy metals in dewatered biosolids and composted biosolids amended clay loam soil.

The exchangeable and water soluble forms of Cu could be available to plants (Shuman, 1991). Phytotoxicity of copper to most plants can occur at about 25 to 40 µg/g dry matter but the normal Cu levels in plants ranges between 5 to 20 mg/kg dry matter (Chaney and Giordano, 1977; Page, 1974). In this study the maximum tissue Cu concentrations of 15.8 and 7.0 µg/g dry matter were recorded at the 65 t/ha biosolids rates for canola and oats tissue respectively and did not affect the growth and performances of either crops.

The uptake of Cu and Zn by plants is strongly regulated by plants (Welch, 1995) and in some cases, plant tissue Cu levels were negatively related with Cu loadings (Heemsbergen, 2009) and the use of bioconcentration factor to examine the relative availability of Cu is a challenge and provide inconclusive results (McLaughlin, 2000).

In this study, the relationship between soil total Cu and Zn residue was poorly related with Cu and Zn levels in canola tissue, particularly in dewatered biosolids treated canola plots. However, in composted biosolids treated plots, soil pH had raised the levels of Cu and Zn in canola and oat tissue.

5.8. Mass balance of Cu and Zn

The mass balance of Cu and Zn in dewatered biosolids amended soil and plants were conducted assuming soil bulk density of 1.24 g/cm^3 and biosolids incorporation depth of 10 cm (in reality the actual biosolids incorporation depth or plough depth is greater). In addition, plant samples were taken at 4-5 leaf stage for canola and 5-6 tillering stage for oats, rather than at harvesting stage and hence the impact of biosolids applications on soil heavy metals levels will considerably be less than shown by this study.

The average crop uptake of Cu and Zn was calculated by multiplying the concentrations of Cu and Zn in plants (mg/kg) by the average biomass yield (kg/ha) for canola and oats.

Hence, the mass balance for Cu and Zn in dewatered biosolids amended canola and oats plots was calculated by taking into account heavy metals residues left after the end of first year crop harvest and the amount of these heavy metals that were taken up by canola and oat crop.

This was conducted by adding up the XRF determined total soil Cu and Zn values in dewatered biosolids amended soil and their corresponding concentration in canola and oats leaf which were extracted using HNO_3/H_2O_2 solution. The data presented in Table 5.22 and Table 23 shows the mass balance of Cu and Zn calculated without subtracting the concentration values of the unamended control plots in the 2006 dewatered biosolids amended canola and oats plots.

Table 5.22 Estimated levels of the accounted and unaccounted losses of Cu and Zn levels in soil and in canola crop after first year applications of dewatered biosolids (kg/ha)

Mass balance of Cu in canola plots						
Biosolids rates (t/ha)	Quantity ofCu applied	Cu residue in soil	Cu uptake	Sum of Cu residue and uptake	Unaccounted balance	Unaccounted balance (%)
0	21.1	17.6	0.039	17.6	3.4	16
5	24.3	18.4	0.029	18.4	5.9	24
25	37.3	23.6	0.135	23.8	13.5	36
45	50.2	31.6	0.218	31.8	18.4	37
65	63.2	37.2	0.284	37.5	25.7	41
Mass balance of Zn in canola plots						
Biosolids rates (t/ha)	Quantity of Cu applied	Cu residue in soil	Plant uptake of Cu	Sum of Cu residue and uptake	Unaccounted balance	Unaccounted balance (%)
0	45.5	36.7	0.24	36.9	8.6	19
5	50.8	41.4	0.17	41.6	9.2	18
25	72.1	45.6	1.01	46.6	25.4	35
45	93.3	62.9	1.67	64.7	28.6	31
65	114.5	68.2	2.08	70.3	44.3	39

Table 5.23 Estimated levels of the accounted and unaccounted losses of Cu and Zn in soil and in oats crop after first year applications of dewatered biosolids (kg/ha)

Mass balance of Cu in oats plots						
Biosolids rates (t/ha)	Quantity ofCu applied	Cu residue in soil	Cu uptake	Sum of Cu residue and uptake	Unaccounted balance	Unaccounted balance (%)
0	21.1	17.26	0.03	17.29	3.8	18
5	24.3	18.74	0.06	18.80	5.5	23
25	37.3	26.10	0.08	26.18	11.1	30
45	50.2	30.44	0.14	30.58	19.7	39
65	63.2	30.48	0.16	30.64	32.6	52
Mass balance of Zn in oats plots						
Biosolids rates (t/ha)	Quantity ofCu applied	Cu residue in soil	Plant uptake of Cu	Sum of Cu residue and uptake	Unaccounted balance	Unaccounted balance (%)
0	45.5	38.1	0.13	38.2	7.4	16
5	50.8	39.7	0.50	40.2	10.6	21
25	72.1	51.7	0.68	52.4	19.6	27
45	93.3	57.2	1.13	58.4	34.9	37
65	114.5	58.4	1.38	59.7	54.8	48

The recovery of Cu and Zn in the unamended control and at the higher dewatered biosolids (45 and 65 t/ha) treated canola plots were relatively similar (Table 5. 22).

Likewise, in the oat plots, the recovery of Cu and Zn in the control and at the highest

(65 t/ha) dewatered biosolids application were not significantly different from each other (Table 5.23).

The observed recoveries of Cu and Zn in the unamended control plots for both canola and oat plots treated with dewatered biosolids were higher than those recorded at the highest dewatered biosolids applied plots. In addition, as dewatered biosolids application increases, a decreasing trend in the recoveries of the heavy metals were also observed, this was because Cu and Zn are associated with organic matter and the decomposition of organic matter through time coupled with the influence of irrigation may have exacerbated and increased the down ward mobility of biosolids Cu and Zn beyond the 10 cm sampling depth.

Similar findings were also reported by Baveye *et al*. (1999) in which case losses of sludge-borne metals ranged from a low of 39 % for Cu and Pb to a high of 60 % of Ni.

Hinesly *et al*. (1984b) based on total heavy metals data reported deficits in metals mass balances. Campbell and Beckett (1988) reported significant increases of Cu and Zn concentrations to a depth of 40-60 cm. Conversely, Brown *et al*. (1997) report the tendency of some metals to move deeper than 80 cm soil depth in long-term plots treated with alkaline sludge products.

The contribution of plant uptake of heavy metals to the mass balance was very low, however, to some extent it is also expected that the HNO_3/H_2O_2 extracting solution was not strong enough to completely dissolve Cu and Zn from canola and oat leaf. In addition to this, the uptake of heavy metals by weeds may have slightly contributed to some of the losses of Cu and Zn from the amended soil. Moreover, the bulk density of the soil was not measured, but approximate value (1.24 g/cm$^{3)}$ was taken, however the density of the soil may or may not be greater than the approximate value used in the computation.

5.9. Predicting the maximum biosolids application rates based on metals

Both biosolids products were added based on NLBAR for the two crops and in 2007 the same application rates were repeated. It is under this assumption that the maximum biosolids application rates to reach EPA Victoria Victoria maximum ceiling limits for Cu and Zn was computed.

Hence, to predict the maximum biosolids application rates and reach the EPA Victoria maximum ceiling limits for Cu and Zn concentrations, equations were estimated from the regression coefficients data presented in Table 5.4 and 5.8. The estimated equations describe the relationship between repeated dewatered biosolids and composted biosolids applications and concentrations of the heavy metals (Cu and Zn) residuals remaining at the end of the two years experiment. Thus, values of the EPA Victoria maximum ceiling limits for Cu and Zn concentrations were inserted into the estimated equations as dependent variable and the possible maximum biosolids application rates were calculated.

The results indicated that the maximum dewatered biosolids that can be applied in canola plots to reach the EPA ceiling limits on average ranged between 381-1345 t dry solids/ha for Cu and 482-635 t/ha for Zn respectively. Similarly, in the oats plots, for Cu and Zn levels not to exceed the EPA Victoria maximum ceiling limits, dewatered biosolids can be applied ranging between 322-1123 t/ha for Cu and 373-490 t/ha for Zn respectively (Table 5.24).

The predicted values for composted biosolids application rates that can be applied in canola plots with out exceeding the EPA maximum ceiling limits for Cu were relatively similar (Table 5.24).

When the two crops were compared, the predicted maximum dewatered biosolids and composted biosolids application rates for canola crop were slightly higher than for the oats.

Indeed, the predicted maximum biosolids application rates calculated for Cu was significantly higher than the corresponding values for Zn, hence the upper limit of dewatered biosolids application rate for Zn (635 t/ha) in canola plots and (490 t/ha) in oats plots were taken to calculate the number of years that dewatered biosolids need to be applied to reach the maximum EPA Victoria ceiling limits for Zn concentrations. Though, it exceeds the N and P requirements of the two crops, particularly for the clay loam soil used in this study, dewatered biosolids could be applied consecutively at 65 t/ha/yr for 10 years in canola plots and 8 years in oats plots. Alternatively, if biosolids were added at agronomic rate (1NLBAR for canola which is equivalent to 10 t ds/ha), dewatered biosolids could be applied consecutively for 64 years. For the oat crop since 1NLBAR was equivalent to 11 t/ha ds, thus dewatered biosolids could be applied for 45 successive years to reach the Victorian EPA maximum ceiling limits for Zn. In practice, biosolids would be applied once in a crop rotation (for reasons of P management), therefore in reality it may take >200 years in total to reach the soil limits. Furthermore, the metal accumulation models are based on a cultivation depth of 10 cm, in practice the plough depth may be 25 cm, so the maximum concentration might not be attained for 500 years in practice (Table 5.24).

Results of the study have shown that even though Cu and Zn concentrations in both biosolids were substantially higher than the soil background concentrations, there were no risks of soil contaminations resulting from elevated levels of Cu and Zn from the applications of these particular biosolids over the two consecutive years.

Table 5.24 The maximum predicted biosolids application rates to reach the EPA Victoria maximum ceiling limits for Cu and Zn levels in biosolids amended canola and oats plots

Heavy metals	Predicting equations	R^2	Residuals at the highest loading rate (mg/kg dm)	Predicted maximum loading rate (t/ha)	EPA Victoria maximum ceiling limits (mg/kg dm)
Dewatered biosolids amended canola and oats plots					
Canola plots					
Cu	y = 0.2074x + 20.98	0.95	45	381-1345	100-300
Zn	y = 0.3268x + 42.49	0.97	82	482-635	200-250
Oats plots					
Cu	y = 0.2498x + 19.40	0.95	49	322-1123	100-300
Zn	y = 0.4287x + 39.99	0.94	90	373-490	200-250
Composted biosolids amended canola and oats plots					
Canola plots					
Cu	y = 0.2122x + 18.22	0.99	48	385-1328	100-300
Zn	y = 0.577x + 39.46	0.99	121	277-364	200-250
Oats plots					
Cu	y = 0.2579x + 15.29	0.99	51	328-1104	100-300
Zn	y = 0.7604x + 32.53	0.98	139	220-286	200-250

The residuals at the highest loading rates refer to the quantity of Cu and Zn remained at the highest dewatered (65 t/ha) and composted (70 t/ha) biosolids treated plots at the end of the two years period, whereas y stands for the analytes Cu and Zn and x stands for dewatered biosolids

5.10. Implications for sustainable utilization of nutrients from biosolids

Sustainable use of biosolids requires the use of nutrients in biosolids at or below the agronomic loading rate and/or use of the soil conditioning properties of biosolids; it also involves protection of human health, the environment and soil functionality (EPA Vic., 2004). In this study, the highest concentrations of total heavy metals (Cu and Zn) observed after two years of successive application of dewatered and composted biosolids did not exceed the Victorian EPA maximum permissible limits. In addition, the levels of Mn, Fe and Pb accumulated in the soil were not high and did not show any negative impact on the performance of either crops or on the receiving soil.

The study clearly showed that the oats-canola cropping sequence had significantly lower residuals of extractable Cu, Zn and Fe in the amended soil after the end of the two year experiment.

The results of the study could be used as inputs to refine and update the Victorian guidelines for land application of biosolids in a clay loam soil with due emphasis given on site specific biosolids applications incorporating crop rotation taking into account soil physical properties particularly pH, CEC, clay content and organic matter.

Dewatered biosolids slightly reduced the pH of the soil at the higher application rates, while composted biosolids raised the soil pH.

The selection of crops based on their heavy metal uptake potential under different crop rotation regime needs to be considered so as to sustainably utilize biosolids nutrients and decrease heavy metals accumulations in the amended soil. The crop rotation may include grasses-legumes and legumes-grasses.

5.11. Conclusion

Application of dewatered and composted biosolids significantly increased the total and DTPA extractable Cu, Zn, Mn, and Fe in the amended soil. However, Total Cu and Zn concentrations determined by XRF even at the highest 65 and 70 t/ha dewatered and composted biosolids amended plots did not exceed the maximum EPA permitted ceiling limits for soils receiving biosolids used for plant production. The study also showed that the concentrations of DTPA extractable soil Zn and Fe residue recorded after two years of the canola-oats rotation were significantly higher than the levels found after the oats-canola cropping sequence, it is expected that the organic acids released by canola roots perhaps decompose the organic matter thus adding soluble metals into the soil. Moreover, the decomposition of canola roots residue might release organic acids into the soil which may change the insoluble metals into soluble forms and hence increase the soluble fractions of the metals in the canola-oats cropping sequence.

This needs further investigation taking into account the effect of rotating canola with cereals on the microbial community and hence the release of microbial nutrients into the soil under glasshouse and field conditions.

Biosolids application significantly affected soil pH, where a decrease and an increase in soil pH were observed following dewatered and composted biosolids applications respectively. Significant positive correlations between DTPA extractable metals and their respective concentrations in plant tissue were also observed, confirming the use of DTPA as reliable estimator of plant available heavy metals in soil.

Among the metals investigated, this study showed that the uptake of Cu and Zn by canola was significantly higher than the control plots and was linear. Uptake of Cu and Zn by oats showed a plateau type response. In addition to this, the concentration of Cu and Zn in canola was considerably higher than the levels observed in oats leaves, hence the use of canola in rotation with other cereal crops on biosolids amended soil may assist in the control of Cu and Zn in biosolids amended soil. However, biosolids application should also consider soil properties such pH, organic matter, CEC and clay content of the biosolids receiving soil.

As a regulatory requirement, periodic monitoring of pH of the biosolids amended soil would be important for the possible prediction of the potential acidifying effect of biosolids. Since soil pH has major effect on metals solubility and their subsequent transfer to plant system. And hence, in sandy acidic soils, if biosolids are applied repeatedly, the total Cu and Zn levels in biosolids amended soils need to be monitored on a regular basis to protect the soil quality. Periodic monitoring of total heavy metals levels in biosolids amended soil would be necessary to check whether heavy metal levels exceed the EPA maximum ceiling limits or not.

The present experiment demonstrated that biosolids can be used for a number of years as a source of plant nutrients with no adverse effect of heavy metals accumulated either in the crops or on the receiving soil.

Although, the biosolids had minor negative impact in terms of heavy metals accumulation in the amended soil and in plants, the field experiments showed that crop rotation in biosolids amended soil may also serve as an option to slightly reduce the impact of DTPA extractable heavy metals residuals that may be added into the soil from the biosolids.

It would be essential to further examine the levels of plant nutrients N, P and S accumulated at various dewatered biosolids and composted biosolids loading rates in the amended soil, and the next chapter discusses the concentrations of N, P and S that remained in biosolids amended soil after two years successive applications of the two biosolids under field conditions. It also describes the levels of N and P uptake by canola and oat crops after each year's crop harvest.

6

CHAPTER 6. CHANGES IN NITROGEN, PHOSPHORUS AND SULFUR IN SOIL AND PLANTS AFTER TWO YEARS SUCCESSIVE APPLICATION OF BIOSOLIDS

Introduction

This chapter discusses the nutrient (N and P) responses observed in 2006 and 2007 before and after rotation of canola and oats in biosolids amended soils and in plants during field trials in which the crops were grown in rotation. It also describes the influence of crop rotation on the levels of N and P residues accumulated in biosolids amended soil.

Total N was analysed by a Leco FP auto carbon and nitrogen analyser, whereas total P was determined by X-ray fluorescence (XRF) spectrometry. The NO_3-N and PO_4-P fractions in biosolids amended soil and plants were determined by flow injection analysis, while NH_4-N was determined by segmented flow analysis. The purpose of such quantification was to assess and evaluate the effect of applications of different rates of dewatered and composted biosolids on the residuals of these nutrients remaining in the amended soil and in plants after each year's crop harvest during the two years period of the field experiment.

The objective was to investigate the effect of various dewatered and composted biosolids applications on total and extractable N and P in soil and in plant tissues and their relationship under a canola and oats cropping regime. The study also examined the effect of biosolids amendment rates on the total sulfur status of a clay loam soil at WWSP Victoria.

6.1. Total Nitrogen in biosolids amended soil

The long term value of biosolids applications could be the provision of supplying slow conversion of organic-N to inorganic-N fractions during the growing season of the crop.

Nitrogen is critically essential for growth of many of the crops grown on biosolids amended soils; it is present in sludge in both organic and inorganic fractions, the quantity being dependent on the method of sewage and sludge treatment processes (Smith, 1992; Smith, 1996; Erhart *et al*. 2005).

Table 6.1 Average values of total nitrogen in ASPAC standard reference materials (STD 75, STD55 and compost 5 for soil and compost, analyzed using Leco FP 2000 auto carbon and nitrogen analyzer

SRMs	Measured (%)		Certified acceptance ranges (%)	
	TN	TC	TN	TC
STD 75	0.101 ± 0.008	1.43 ± 0.08	0.085 - 0.145	1.14 - 1.56
STD 55	0.122 ± 0.005	2.53 ± 0.003	0.097 - 0.177	1.7 - 2.9
Compost 5	0.545 ± 0.016	46.1 ± 0.8	0.5526 - 0.6486	43.63 - 47.27

STD 75 and STD 55 stands for soil and compost 5 for compost standard reference materials. The measured values indicate mean and standard deviation of triplicate measurements
ASPAC: Australian Soil and Plant Analysis Council.

6.1.1. Data validation and analytical accuracy

Australian Soil and Plant Analysis council (ASPAC) standard reference materials (STD 75, STD55 and compost 5 for soil and compost) were analysed for total nitrogen and total carbon concentrations as a measure to validate the accuracy of the results obtained from Leco FP 2000 auto carbon and nitrogen analyser.

Table 6.1 shows the average values of ASPAC standard reference materials (STD 75, STD55 and compost 5 for soil and compost, STD143 and STD63 tea and eucalyptus leaves for plants reference materials analysed using Leco FP 2000 auto carbon and nitrogen analyser. The measured values for total nitrogen and carbon in soil and total nitrogen in plant standard reference materials were all in the certified acceptance ranges indicating good agreement.

6.1.2. Changes in TN in dewatered biosolids and composted biosolids amended soil

Table 6.2 shows the levels of total-N, NH_4-N, NO_3-N and total- C in soil and biosolids before the start of the experiment (June 2006). The total-N pool and NH_4-N levels contained in dewatered biosolids were considerably higher than composted biosolids; however, as expected composted biosolids had greater NO_3-N concentrations than dewatered biosolids.

Table 6.2 Concentrations of total and extractable N in soil and in the biosolids used for the experiments.

Analytes	Soil	Dewatered biosolids	Composted biosolids
Total- N %	0.17 ± 0.002	4.22 ± 0.005	1.44 ± 0.003
NH_4-N(μg/g)	5.1	3740	na
NO_3-N (μg/g)	2.9	830	1864

Total N was determined by Leco FP auto carbon and nitrogen analyzer, whereas NO_3-N and NH_4-N were analyzed by flow injection analyzer (FIA) and segmented flow analysis (SFA), respectively

Biosolids amended soil samples were taken after the end of each year crop harvest (in 2006 and 2007) and analysed to examine the impacts of dewatered and composted biosolids loading rates on the nitrogen pool in the soil during the two years period. The results are shown in Figure 6.1.

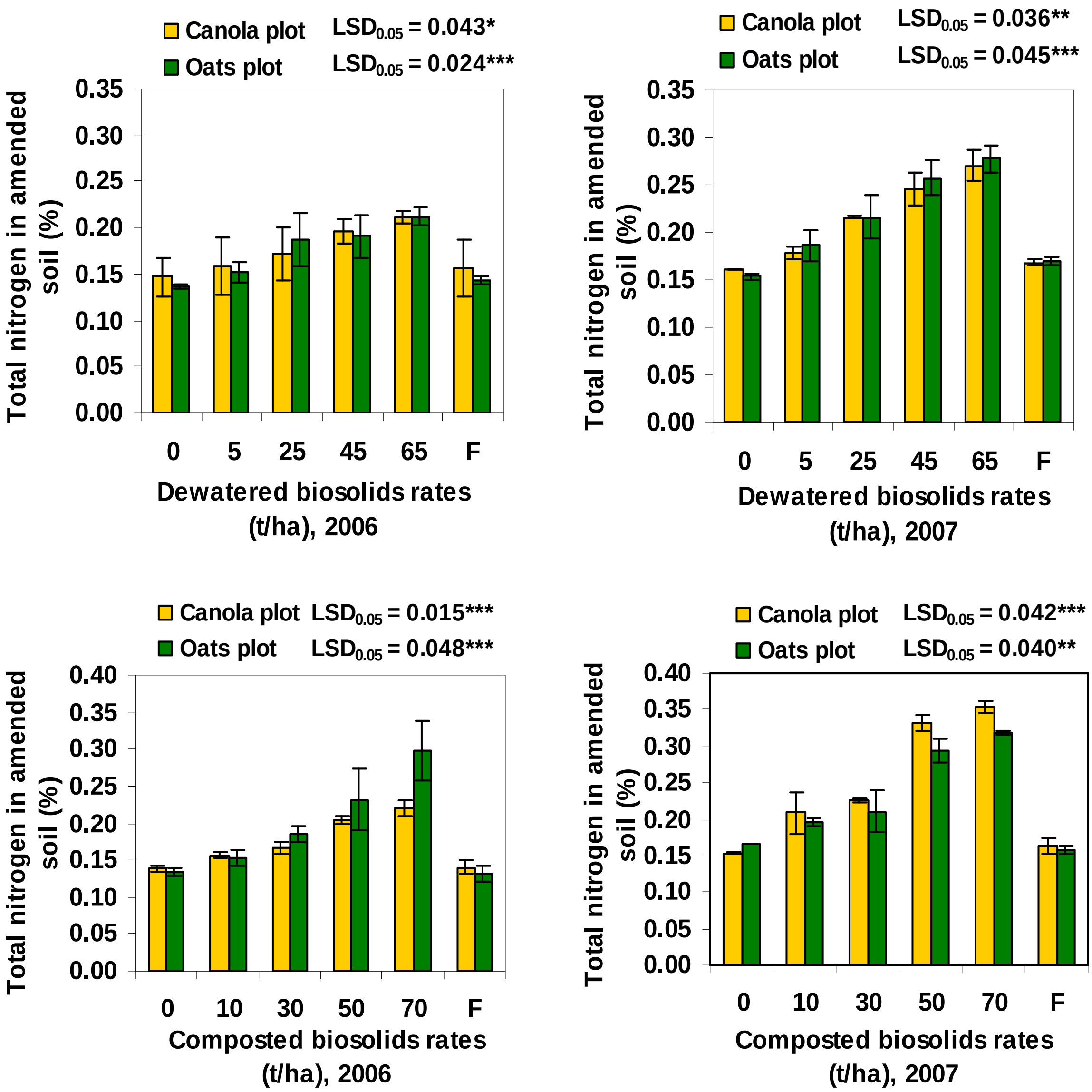


Figure 6.1 The change in the mean values of soil TN in canola and oats plots under increasing applications rates of dewatered biosolids and composted biosolids in 2006-2007 field experiments.

Table 6.3 Concentration of TN (total nitrogen) in dewatered and composted biosolids amended soil samples taken after first and second crop harvest from canola and oats plots

Effect of dewatered biosolids amendment on TN (in 2006 and 2007)				
Biosolids rates (t/ha)	Amended soil samples from canola plot		Amended soil samples from oats plot	
	TN (%) 2006	TN(%) 2007	TN (%) 2006	TN (%) 2007
0	0.15 (0.02)	0.16 (0.0001)	0.14 (0.001)	0.15 (0.0001)
5	0.15 (0.03)	0.18 (0.01)	0.15 (0.01)	0.19 (0.001)
25	0.18 (0.03)	0.22 (0.001)	0.19 (0.03)	0.22 (0.02)
45	0.19 (0.01)	0.26 (0.02)	0.19 (0.02)	0.26 (0.02)
65	0.21 (0.01)	0.28 (0.02)	0.21 (0.01)	0.28 (0.02)
Fertilized	0.15 (0.03)	0.17 (0.0001)	0.14 (0.00)	0.17 (0.01)
$LSD_{0.05}$	0.043*	0.036**	0.024***	0.045**
Effect of composted biosolids amendment on TN in soil (2006 and 2007)				
0	0.14 (0.0001)	0.15 (0.0001)	0.14 (0.01)	0.17 (0.0001)
10	0.16 (0.01)	0.21 (0.03)	0.15 (0.01)	0.20 (0.001)
30	0.17 (0.01)	0.23 (0.0001)	0.19 (0.01)	0.21 (0.03)
50	0.20 (0.01)	0.33 (0.01)	0.23 (0.04)	0.29 (0.02)
70	0.22 (0.02)	0.35 (0.01)	0.30 (0.04)	0.32 (0.00)
Fertilized Plot	0.14 (0.01)	0.16 (0.01)	0.13 (0.01)	0.16 (0.01)
$LSD_{0.05}$	0.0151***	0.042***	0.048***	0.040**

Figures in parenthesis indicate standard deviation f triplicate measurements whereas, *, **, *** and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$ $p < 0.001$ level respectively

Year 1

Figure 7.1 shows the variation in total N for each biosolids type, on each crop for each year.

In the 2006 biosolids plot trial (before rotation), dewatered biosolids application had a significant ($p < 0.05$ and $p < 0.001$) effect on the level of soil total N, changing on average from 0.15 to 0.21 % in canola and from 0.14 to 0.21 % in oats plots respectively in the plots with the highest biosolids loading rates.

Composted biosolids applications in 2006 also significantly increased the level of soil total N ($p < 0.001$) changing from 0.14 to 0.22 % in canola and from 0.14 to 0.30 % in oat plots respectively (Fig.6.1).

Year 2

In 2007, reapplying dewatered biosolids at the same rate as 2006 resulted in an increase in soil total N from 0.16 to 0.28 % in canola and from 0.15 to 0.28 % in oats plots ($p < 0.01$). The maximum percent total N in amended soil was recorded at the highest (65 t/ha) dewatered biosolids loading rate.

The effect of repeated application of dewatered biosolids in 2007 (after rotation) increased the level of total N in the amended soil by 0.07% in canola and by 0.07 % in oats plots ($p < 0.01$). There was no significant difference ($p > 0.05$) in residual total N in the soil between canola and oats plots receiving dewatered biosolids.

The application of composted biosolids increased the level of total N ($p < 0.001$) changing from 0.15 to 0.35 % for canola and from 0.17 to 0.32 % for oats plots respectively. The highest soil total N was recorded in the 70 t/ha composted biosolids treated plots. However, repeated application of composted biosolids had similar effect in which case total N increased by 0.2 % and by 0.15 % in canola and oats plots respectively.

The effect of canola-oats and oats-canola cropping sequence on the level of soil total N was compared in terms of the net total N levels accumulated at the end of the two years experiment and the results are summarized in Table 6.4.

Table 6.4 The effect of cropping sequence on the level of soil total N residuals accumulated in dewatered and composted biosolids amended canola and oats plots at the end of the two years experiment (expressed in mg/kg)

Cropping sequence in dewatered biosolids treated plots							
Analytes	Yr 1 canola	Yr 2 oats	Yr 2 - Yr 1	Analytes	Yr 1 oats	Yr 2 canola	Yr 2 – Yr 1
Total N	600	1300	700	Total N	700	1200	500
Cropping sequence in composted biosolids treated plots							
Analytes	Yr 1 canola	Yr 2 oats	Yr 2 – Yr1	Analytes	Yr 1 oats	Yr 2 canola	Yr 2 – Yr 1
Total N	800	1500	700	Total N	1600	2000	400

Dewatered biosolids and composted biosolids amended soil samples were taken after each year crop harvest and total N was determined using Leco FP auto carbon and nitrogen analyzer.
Concentrations of soil total-N residue left at the highest 65 t/ha and 70 t/ha dewatered biosolids and composted biosolids treated plots was subtracted from the unamended control plots to compare the effect of cropping sequence on N residues accumulated in soil.

When the amount of total N left at the end of year 1 was subtracted from the amount of N remained at the end of year 2, in both dewatered and composted biosolids treated plots slightly lower total N was observed for oats-canola rotation than the levels found in the canola-oats cropping sequence (Table 6.4).

6.2. NO_3-N residue in biosolids amended soil

Nitrification is the aerobic conversion of ammonium (NH_4- N) into nitrite (NO_2^-) and nitrate (NO_3-N) by nitrifying bacteria. The conversion of NH_4- N to NO_3-N by Nitrosomonas and Nitrobacter bacteria enhances the mobility of N in soil (because of the dominance of negatively charged surfaces on soil colloids), potentially increasing N availability to plants, but also increasing the loss of NO_3-N via leaching. The process of nitrification has a net acidifying effect on the soil and the resulting drop in soil pH which may alter the availabilities of other plant nutrients (Paul and Clark 1996). The nitrification process (oxidation of NH_4-N into NO_3 by nitrifying bacteria) as described by Geradi, (2002) is represented by the equation:

$$NH_4 - N + 1.5O_2 \xrightarrow{Nitrosomonas} NO_2^- + H_2O + 2H^+ + energy$$

$$NO_2 + 0.5O_2 \xrightarrow{Nitrobacters} NO_3^- + energy$$

Biosolids amended soil samples which were taken during summer season after each year 2006 and 2007 crop harvest were analysed to examine the impacts of different biosolids applications rates on the level of NO_3-N residues accumulated after each year's application of the two biosolids types.

6.2.1. Validation of analytical data for NO_3-N in biosolids amended soil

Concentrations of NO_3-N and NH_4-N in biosolids amended soil were extracted using 2 M KCl solution (Keeny and Nelson, 1982) and NO_3-N was analysed by flow injection analysis using the cadmium reduction method (Technicon Instrument Corporation, 1971), whereas NH_4-N was determined by segmented flow analysis utilizing the Berthelot reaction according to the phenate method (Searle, 1984).

To validate the accuracy of soil NO_3-N data, soil extracts were spiked with a known concentration of NO_3-N standards ranging from 0.8 to 1.6 µg/g and analysed in duplicate. The percent recoveries of the spiked samples compared with the unspiked samples were on average 94 % for the amended soil (Table 6.5).

The precision of the soil extraction procedures for NO_3-N analysis were also evaluated by extracting and analysing five replicate soil and plant samples. The coefficient of variation was 2.4 % for soil indicating good precision of the extraction procedure.

Obviously, while carrying out flow injection analysis, varying flow rates, temperature change and inefficient reduction of the sample in the cadmium column may lead to biased results, hence instrument drift was checked by analysing the highest NO_3-N standard (1.6 µg/g) at the beginning and end of the analysis and the recovery on average was 98.8 % indicating only a minor 1.2 % instrument drift.

Table 6.5 The recovery of NO_3-N in 2M KCl soil extracts and analyzed by flow injection analysis using the cadmium reduction technique.

Soil NO_3-N			
	Measured concentrations (mg/L)		
Spiked concentration (mg/L)	Unspiked sample	Spiked sample[a]	Recovery (%)
1.6	0.12 ± 0.02	1.53 ± 0.03	88
0.8	0.26 ± 0.00	1.03 ± 0.00	96.2
0.8	0.155	0.95 ± 0.04	99.0

Two spiking procedures were followed, first, 2 mL of the 20 mg/L NO_3-N standards was taken and spiked in 50 mL volumetric flask (0.8 mg/L NO_3-N) and analyzed and the percent recovery was calculated, the second procedure was that 2ml of the 20 mg/L NO_3-N was taken and spiked in 25 mL volumetric flask (1.6 mg/L NO_3-N) containing soil extracts and analysed the percent recovery determined

6.2.2. Changes in NO_3-N in biosolids amended soil

Year 1

In the 2006 trial, when the control and the highest dewatered biosolids treated plots (65 t/ha) after crop harvest were compared, NO_3-N levels significantly increased from 0.06 to 1.78 mg/kg in canola plots and from 6 to 21 mg/kg in the oats plot ($p < 0.001$).

Composted biosolids loading rates also significantly increased the NO_3-N levels from 4.4 to 13 mg/kg in the canola plots and from 2.4 to 4.0 mg/kg in the oat plots (Fig.6.2). Statistical analysis of the soil residual NO_3-N of the two crops showed a significant difference between canola and oats plots treated with dewatered biosolids in which 13 mg/kg more NO_3-N was recorded in the soils from the oat plots than from canola plots.

Comparing the influence of the two biosolids types, 6.85 mg/kg more NO_3-N was observed in canola plots treated with composted biosolids than in the oats plots, thus overall more NO_3-N was found in the oats plots than in canola plots (Fig.6.2).

Year 2

In the 2007 trial, repeated application of dewatered biosolids increased the concentration of NO_3-N from 9 to 57 mg/kg in the canola plots and from 9 to 56 mg/kg in the oat plots. There was also a similar increase for composted biosolids treated canola plots changing from 10 to 60 mg/kg but quite a different response in the oat plots increasing from 11 to 17 mg/kg respectively .

There was no significant difference in terms of soil NO_3-N between canola and oats plots treated with dewatered biosolids. However, 43 mg/kg more NO_3-N was recorded in canola plots treated with composted biosolids than in the oats plots.

There was no significant difference in the level of soil NO_3-N between canola plots treated with dewatered and composted biosolids, but a 41 mg/kg more NO_3-N was recorded in oats plots treated with dewatered biosolids than composted biosolids treated oats plots (Fig.6.2).

In the 2006 and 2007 composted biosolids amended oats plots, the soil NO_3-N residue levels were very low and this was not expected, however the possible reason for this results would be, after sampling the composted biosolids amended soils, the samples were left for longer period of time during the air drying procedure and NO_3-N was the most unstable element and therefore significant proportion of the NO_3-N component might have been lost through denitrification or ammonia volatilization.

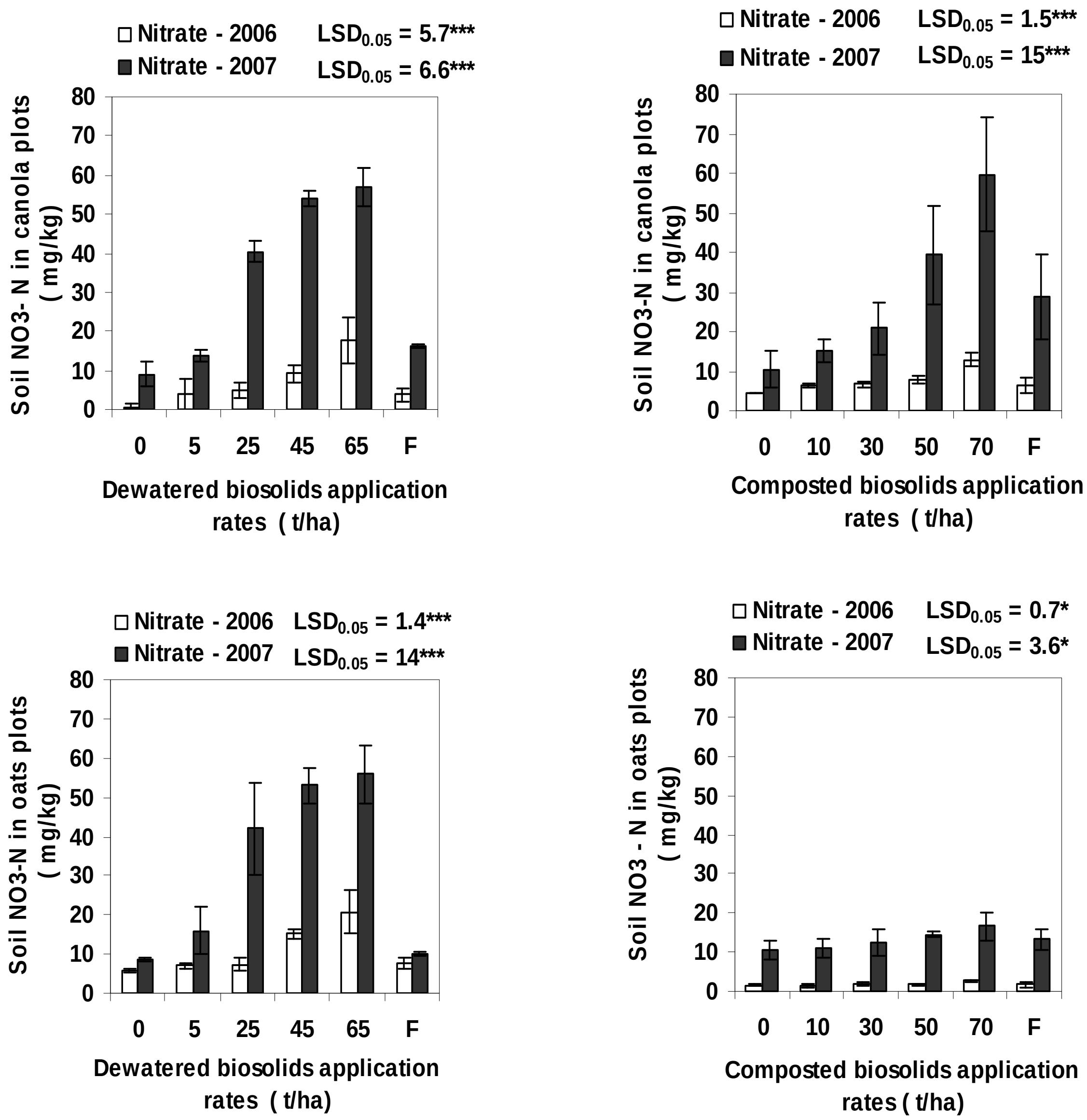


Figure 6.2 The effect of dewatered and composted biosolids application rates on the level of NO_3-N accumulated on the top 10 cm of soil in canola and oats plots in 2006 and 2007 field experiments. The LSD $_{0.05}$ values refers to the least significant difference (t-test) between the mean values of NO3-N at 5 % probability, whereas ** and *** refers to significant treatment effects in ANOVA (F-test) at $p < 0.01$ and $p < 0.001$ levels respectively.

6.3. Leaching of NO_3-N down the soil profile

The leaching of NO_3-N following biosolids application in most cases could potentially contaminate the ground water, thus NO_3-N leaching can be a limiting factor for long term feasibility of agricultural land application of biosolids (Polgase and Robinson, 1996).

High compost application rates (90 t/ ha fresh weight) have been shown to increase leaching of NO_3-N (Mamo *et al.*, 1999). For this reason, the concentrations of NO_3-N possibly leaching down the soil profile in the amended soil using only the highest biosolids treated plots was investigated and presented for each biosolids and crop types.

Comparisons of NO_3-N levels at various soil depths were made between the unamended control and the highest (65 and 70 t/ha) dewatered biosolids and composted biosolids receiving plots. Comparisons were also made between biosolids and crop types during the two years of the field experiment.

Year 1

In 2006, concentrations of NO_3-N recorded at various soil depths for the highest (65 t/ha) dewatered biosolids amended canola and oats plots were significantly ($p < 0.001$) higher than the levels observed in the unamended control plots. The highest soil NO_3-N concentrations of 45 and 50 mg/kg were recorded in the top 0-20 cm of soil for both canola and oats plots.

The maximum soil NO_3-N levels in the control plots (8.6 and 10 mg/kg) were recorded at the 60 and 40 cm soil depths. As soil depth increased, a decreasing trend in the level of soil NO_3-N was noted and most of the soil NO_3-N levels were accumulated in the 20-60 cm soil depth.

When the control plot was compared with the highest dewatered biosolids rates (65 t/ha), the concentration of NO_3-N in the 0-20 and 20-40 cm soil depths were 10 and 4 fold greater for canola and 11 and 5 fold greater for oats plot respectively (Fig 6.3).

Year 2

In the 2007 dewatered biosolids trial, NO_3-N levels recorded at the various soil depths of the highest (65 t/ha) dewatered biosolids amended canola and oats plots were significantly ($p < 0.001$) higher than the unamended control plots. The highest soil NO_3-N concentrations (64 and

136 mg/kg) were observed in the top 20 cm for the canola and oats plots respectively, indicating a significantly ($p < 0.001$) higher amount of NO_3-N was accumulated in oats plots than in canola plots. When the control plot was compared with the highest dewatered biosolids rates (65 t/ha), the level of NO_3-N in the 40-60 cm soil depth was 13 and 45 fold greater for canola and oats plots respectively.

In the composted biosolids trial, NO_3-N levels recorded at the highest (70 t/ha) treated canola plots were statistically different ($p < 0.001$) from the control plots.

As NO_3-N release from compost is usually small, the composted biosolids treated oat plots were not significantly different from the control plots (Fig 6.3 and Appendix H Table H-1), this was not unexpected, however as described above under section 6.2.2, the soil samples taken from composted biosolids amended oats plots were left for longer period during the air drying process and NO_3-N may have lost through various routes.

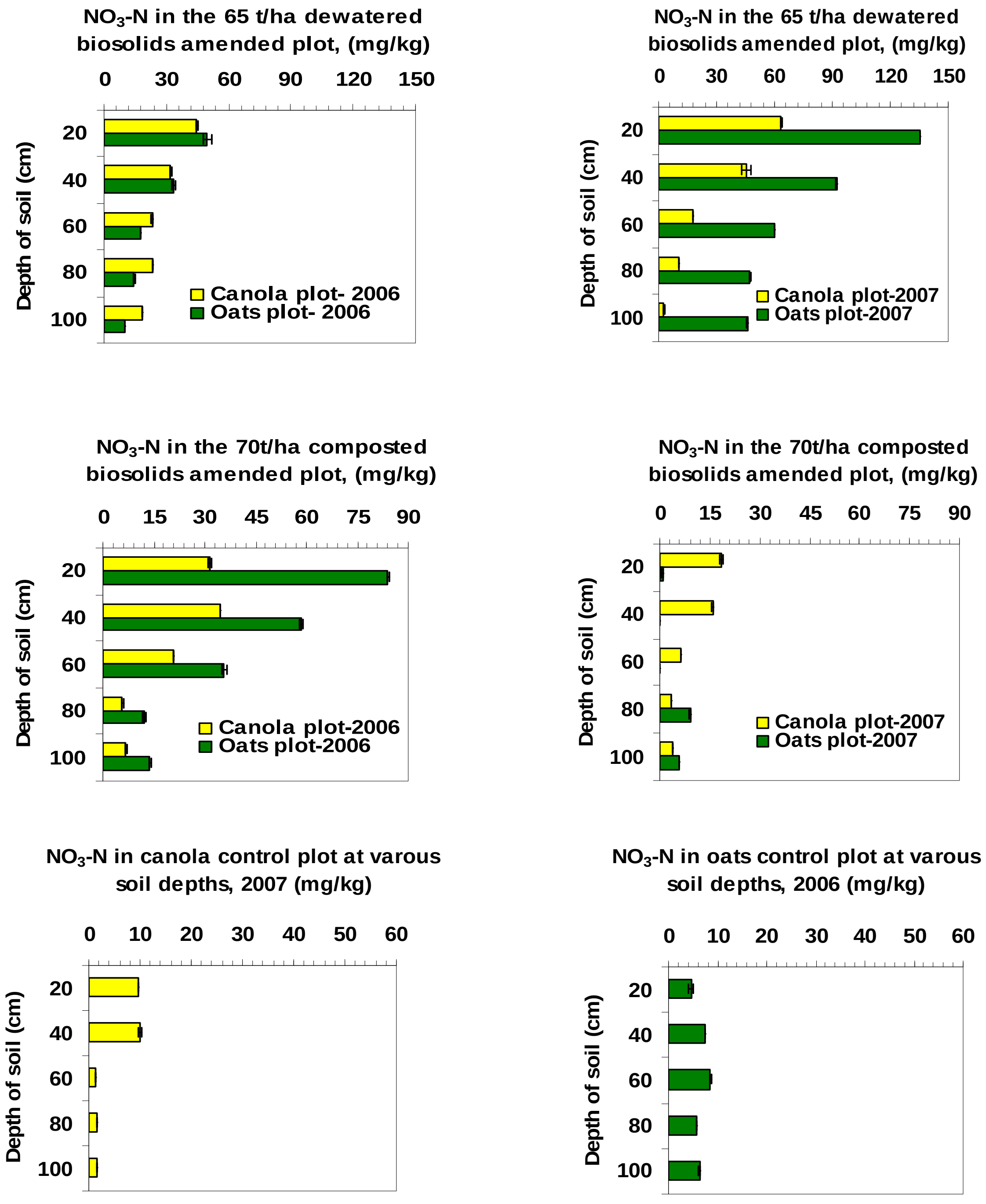


Figure 6.3 Leaching of NO_3-N at various soil depths for the highest application rates of 65 and 70 t/ha dewatered and composted biosolids amended soils compared with unamended controls plots, taken from canola and oats plots in 2006 and 2007 field experiments. The error bars represent standard deviations of triplicate measurements.

6.4. The influence of applied biosolids on total N in plant tissue

Plant analysis has been developed primarily to provide useful information on the nutrient status of plants as a guide to nutrient management for optimum crop production. Increasingly, it is being used to safeguard the environment from over fertilization of crops and pastures (Reuter and Robinson, 1997).

Total N in plant tissue was analysed to examine the effect of biosolids and crop types on the level of total N uptake by canola and oats crops. Canola and oats leaves were analysed for total N concentration at particular growth stages of the plants and the results are described below:

6.4.1. Data validation and analytical accuracy

Table 6.6 shows the analytical results of ASPAC plant standard reference materials (STD143 and STD 63 tea and eucalyptus leaves) analysed for total nitrogen using Leco FP 2000 auto carbon and nitrogen analyser. The measured values for total nitrogen in plant standard reference materials were all in the certified acceptance ranges indicating good agreement.

Table 6.6 Recovery of ASPAC plant standard reference materials analyzed for total nitrogen using Leco FP auto carbon and nitrogen analyzer to validate the total nitrogen data for canola and oats shoots.

Standard reference materials	Measured values TN (%)	Certified acceptance ranges TN (%)
STD 143	4.190 ± 0.010	3.74 - 4.73
STD 63	0.996 ± 0.001	0.866 - 1.194

STD 143 and STD 63 stand for Tea and Eucalyptus leaves standard reference materials. The measured values indicate mean and standard deviation of triplicate measurements.
ASPAC: Australian Soil and Plant Analysis Council.

6.4.2. Changes in total N in plant tissue due to biosolids application

Year 1

As would be expected, the highest level of plant total N in canola and oats tissue in the 2006 plot trial was observed at the 65 t/ha dewatered biosolids application rate.

Plant leaf analysis in the 2006 plot trial showed that dewatered biosolids rate (65t/ha) significantly increased ($p < 0.01$) the level of TN in canola and oats plant matter from 5.7 to 7.1 and from 2.2 to 4.9 %, respectively.

In the 2006 trial the net total N accumulated due to dewatered biosolids application in plant matter was 1.48 and 2.27 % for canola and oats crops respectively.

However, composted biosolids loadings rates had no significant effect on the level of TN in canola and oats plant matter (Fig 6.4).

Year 2

In the 2007 trial, both biosolids types were reapplied at the same rate as in 2006 and TN significantly increased changing from 5.0 to 7.2 % in canola leaves and from 3.3 to 4.1 in oat leaves when treated with dewatered biosolids. A similar increase (from 5.1 to 6.8 %) was observed in canola leaves treated with composted biosolids; nevertheless, the effect of composted biosolids on TN in oat leaves was not significant.

Hence, in 2007 the net total N accumulated in plant matter due to dewatered biosolids application was 2.14 and 0.83 % for canola and oats crops respectively (Fig 6.4).

Table 6.7. The effect of dewatered biosolids and composted biosolids applications on the levels of total nitrogen (%) in canola and oat leaves in 2006 and 2007 field experiments.

Dewatered biosolids (t/ha)	2006		2007	
	TN in canola leaf	TN in oats leaf	TN in canola leaf	TN in oats leaf
0	5.7 ± 0.3	2.2 ± 0.3	5.0 ± 0.7	3.3 ± 0.3
5	5.8 ± 0.4	2.9 ± 0.6	5.7 ± 0.4	3.0 ± 0.3
25	6.6 ± 0.3	3.0 ± 0.2	6.4 ± 0.1	3.9 ± 0.3
45	6.9 ± 0.1	3.6 ± 0.3	6.8 ± 0.1	4.3 ± 0.1
65	7.1 ± 0.1	4.9 ± 0.3	7.2 ± 0.3	4.1 ± 0.2
Fertilized	6.6 ± 0.4	3.2 ± 0.1	6.4 ± 0.4	3.7 ± 0.3
$LSD_{0.05}$	0.42**	0.63**	0.70**	0.54**
Composted biosolids (t/ha)	2006		2007	
	TN in canola leaf	TN in oats leaf	TN in canola leaf	TN in oats leaf
0	5.4 ± 0.4	2.4 ± 0.2	5.1 ± 0.2	3.4 ± 0.4
10	5.0 ± 0.1	2.6 ± 0.5	5.7 ± 0.5	3.3 ± 0.4
30	5.4 ± 0.3	2.9 ± 0.9	6.4 ± 0.1	3.7 ± 0.2
50	5.6 ± 0.5	3.2 ± 0.3	6.4 ± 0.3	3.9 ± 0.2
70	5.4 ± 0.5	3.3 ± 0.2	6.8 ± 0.6	4.2 ± 0.4
Fertilized	5.5 ± 0.2	3.6 ± 0.9	6.2 ± 0.4	4.4 ± 0.3
$LSD_{0.05}$	ns	ns	0.62**	ns

Values indicate means of triplicate measurements, whereas LSD values refers to the least significant difference (t-test) between the mean values of TN in plant matter at 5% probability, whereas the superscripts *,**, *** and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.01$ and $p < 0.001$ levels and not significant respectively.

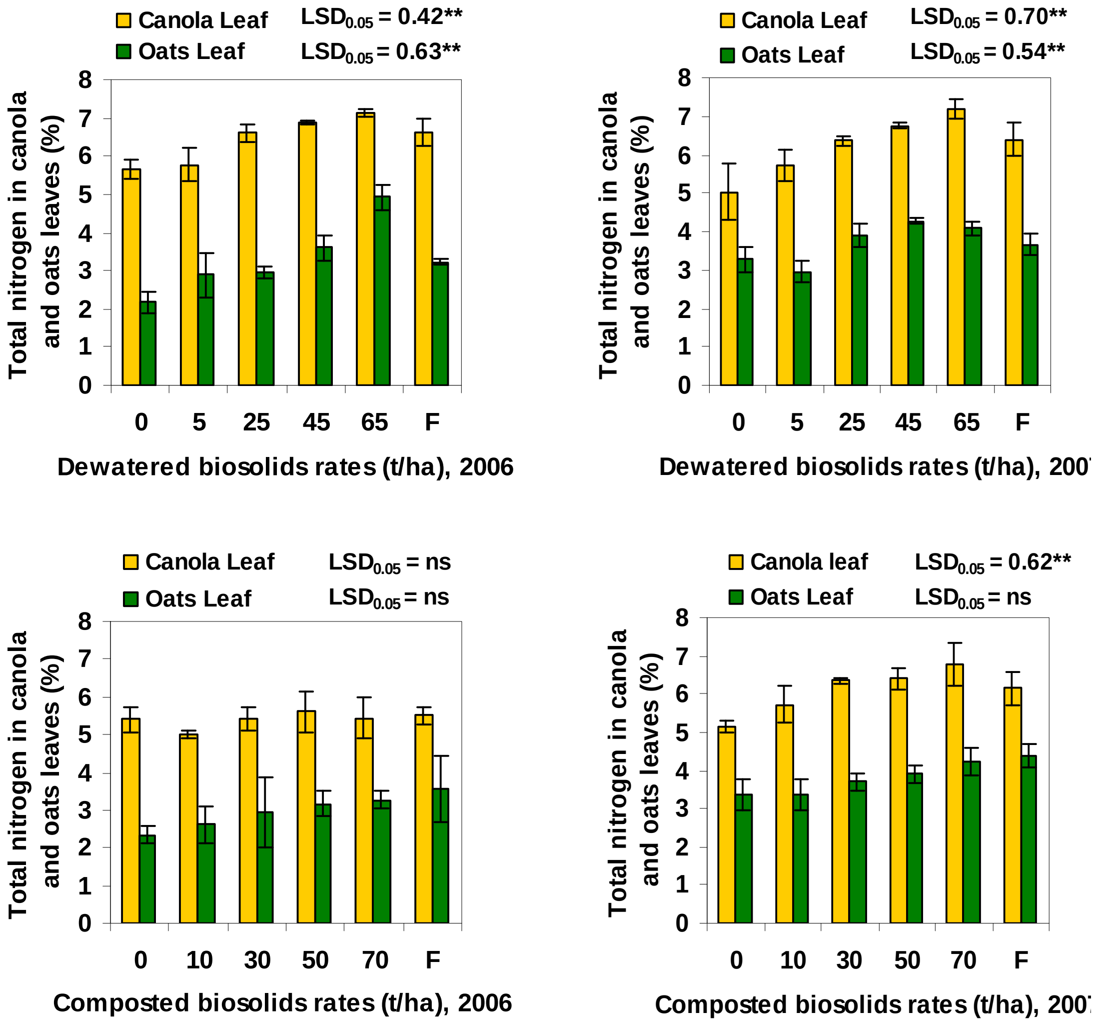


Figure 6.4 Graphical representation of levels of total nitrogen in canola and oats plant matter resulted from different application rates of two different sources of biosolids in 2006 and 2007 field experiments. The error bars represent standard deviations of triplicate measurements. LSD $_{0.05}$ values refers to the least significant difference (t-test) between the mean values of TN in plant matter at 5% probability, whereas **, *** and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.01$ and $p < 0.001$ levels and not significant respectively.

6.5. Effect of applied biosolids on NO_3-N in plant tissue

Nitrate concentration in acetic acid extracts of fresh plant parts has been used for assessing the N status of vegetable crops, grapes and other crops in California (Geraldson *et al.*, 1983).

To assess the effect of dewatered biosolids and composted biosolids applications on the nitrogen status of the canola and oats crops, plant samples for canola at 4-5 leaf stage and for oats at 5-6 tillering stages were taken and the NO_3-N concentration in ground plant matter was extracted using 2 % acetic acid solution and analysed by the cadmium reduction method using flow injection analysis (as described in chapter 3 section 3.4.1). Results of the analytical data together with data validation procedures are presented in Table 6.8 and 6.9 below.

6.5.1. Data validation and analytical accuracy

To validate the plant NO_3-N data, plant extracts were spiked with a known concentration of NO_3-N standards ranging from 0.2 to 0.4 μg/g and analysed in duplicate. The percent recoveries of the spiked samples compared with the unspiked samples were on average ranged 100-116 % for plants (Table 6.8).

The precision of the plant extraction procedures for NO_3-N analysis was also evaluated by extracting and analysing five replicate plant samples. The coefficient of variations was 3.1% for plants indicating good precision of the extraction procedure.

Table 6.8 The recovery of NO_3-N extracted in 2 % acetic acid and analyzed by flow injection analyzer using the cadmium reduction method.

Experiments	Spiked concentration (mg/L)	Measured concentrations (mg/L)		Recovery (%)
		Unspiked sample	Spiked sample[b]	
NO_3-N in canola and oat leaves , 2006				
Exp.1	0.2	0.038	0.237 ± 0.008	99
Exp.2	0.4	0.5975	0.9925 ± 0.0	99
Exp.3	0.4	0.016	0.397 ± 0.0	95
Exp.4	0.4	0.0148	0.4437 ± 0.012	107
NO_3-N in canola and oat leaves , 2007				
Exp.1	0.4	0.0336	0.5232 ± 0.002	122
Exp.2	0.4	0.1933	0.6638 ± 0.0004	117
Exp.3	0.4	0.3945	0.8514 ± 0.004	114
Exp.4	0.4	0.1021	0.5490 ± 0.0005	112

Plant extracts were spiked with 1 mL of the 20 mg/L NO_3-N standards in 50 mL volumetric flask (0.4 mg/L NO_3-N) and analyzed and the percent recovery was calculated, in experiment 1 (for canola leaf samples taken from dewatered biosolids treated plots), plant extracts were spikes with 1 mL of the 20 mg/L NO_3-N standards in 100 mL volumetric flask (0.2 mg/L NO_3-N) and analyzed and the percent recovery was calculated.

In the 2006 trial, Experiment 1 and 2 refers to plant samples taken from canola plots treated with dewatered and composted biosolids, whereas, Experiment 3 and 4 refers to plant samples taken from oats plots treated with dewatered and composted biosolids respectively.

b: Values indicate mean ± sd of duplicate measurements for each experiments, however, unspiked plant extracts in 2006 and 2007 were not analyzed in duplicate.

6.5.2. Changes in NO_3-N in plant tissue

Year 1

Dewatered biosolids application changed the concentration of NO_3-N in both canola and oats tissue. In the 2006 trial, the levels of NO_3-N in plant tissue changed from 784 to 5100 µg/g for canola and from 86 to 7542 µg/g for oats crops. On the other hand, composted biosolids application increased the level of NO_3-N in canola tissue from 257 in the control plot to 1650 µg/g in the 50 t/ha composted biosolids treated plot, but the change in NO_3-N in composted biosolids treated oats tissue was not significantly different from the levels found in the control oat crops.

When the effect of biosolids types on plant NO_3-N levels were compared, the net concentration of NO_3-N recorded (4316 µg/g) in canola tissues treated with dewatered biosolids in the 2006 trial was significantly ($p < 0.001$) higher than the corresponding levels recorded (1393 µg/g) in canola tissues treated with composted biosolids. Slightly more NO_3-N concentrations in oats tissues were observed than in canola tissues (Table 6.9).

Table 6.9 The effect of dewatered biosolids and composted biosolids applications on the levels of NO_3-N (µg/g) in canola and oats leaves in 2006 and 2007 field experiments.

Dewatered biosolids (t/ha)	Canola leaf NO_3-N	Oats leaf NO_3-N	Composted biosolids (t/ha)	Canola leaf NO_3-N	Oats leaf NO_3-N
2006 field experiments					
0	784 ± 388	85 ± 5	0	257 ± 24	na[b]
5	2470 ± 1869	445 ± 39	10	389 ± 8	na
25	5732 ± 100	607 ± 74	30	985 ± 638	na
45	4723 ± 377	832 ± 45	50	1649 ± 821	na
65	5100 ± 259	7542 ± 848	70	747 ± 926	na
Fertilized	5629	814 ± 419	Fertilized	3093 ± 779	na
$LSD_{0.05}$	1596***	721***	$LSD_{0.05}$	894*	na
2007 field experiments					
0	629 ± 533	270 ± 6	0	414 ± 179	333 ± 40
5	1156 ± 86	491 ± 10	10	795 ± 340	219 ± 111
25	2624 ± 148	5561 ± 518	30	2766 ± 383	1597 ± 538
45	6578 ± 919	6272 ± 437	50	5041 ± 31	1504 ± 137
65	9154 ± 589	7024 ± 689	70	8027 ± 1327	2516 ± 330
Fertilized	2942 ± 476	2535 ± 520	Fertilized	5790 ± 1959	2714 ± 42
$LSD_{0.05}$	612***	763***	$LSD_{0.05}$	1168***	1149***

The superscripts *,**,*** and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant, respectively, whereas na stands for concentrations not available. The letter 'b' indicates the analytical data for the 2006 NO_3-N concentrations for the oats leaves were not retrieved due to problem with the FIA instrument as the result data are not available.

Year 2

In the 2007 trial, the application of dewatered biosolids had a similar effect with the 2006 observations changing plant tissue NO_3-N levels from 629 to 9154µg/g for canola and from 270 to 7024 µg/g for oats tissue ($p < 0.001$). Whereas, in the 2007 composted biosolids treatments, NO_3-N increased from 414 to 8027 µg/g in canola and from 333 to 2516 µg/g in oats tissues respectively. As shown in Figure 6.5 NO_3-N in both canola and oats tissue showed an increasing trend following dewatered and composted biosolids application rates.

The levels of NO_3-N observed in canola tissue were significantly higher than the corresponding levels observed in oats tissue (Table 6.9).

When the 2006 and 2007 tissue NO_3-N levels were compared, there was a net increase of 4209 µg/g NO_3-N in canola tissue at the 65 t/ha treated plots due to repeated application of dewatered biosolids. However, in 2007, at the 25 and 45 t/ha application rates, NO_3-N levels in oats tissues was significantly higher than the 2006 recordings.

The net change in NO_3-N levels in canola tissue due to repeated application of composted biosolids was 6221 µg/g for canola and 2516µg/g for oats respectively.

Similarly, in the 2007 experiment, when the effect of biosolids types on plant tissue NO_3-N levels were compared, the concentration of NO_3-N recorded (8525 µg/g) in canola tissue treated with dewatered biosolids was significantly ($p < 0.05$) higher than the corresponding levels recorded (7614 µg/g) in canola tissues treated with composted biosolids, hence dewatered biosolids had greater effect in changing plant NO_3-N levels than composted biosolids in both years of the trial for both crops.

From the results presented above, the concentration of NO_3-N in canola tissue treated with dewatered biosolids and composted biosolids was significantly higher than those observed in oats tissues suggesting NO_3-N uptake by canola was higher than oats crops (Fig. 6.5 and Table 6.9).

The maximum NO_3-N levels in canola and oats tissues were recorded at the highest (65 t/ha) dewatered biosolids application rate.

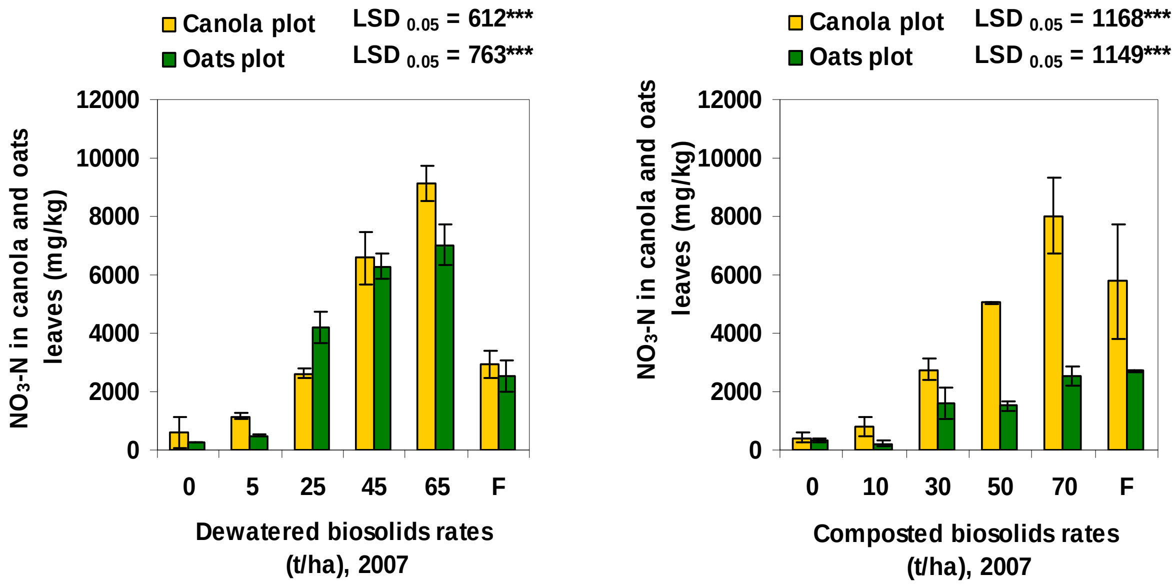


Figure 6.5 Nitrate uptake by canola and oats in dewatered and composted biosolids amended soil in the 2007 field experiment. The superscript *** refers to significant treatment effects in ANOVA (F-test) at $P < 0.001$, whereas LSD $_{0.05}$ stands for the least significant difference between the mean values of NO_3-N in canola and oats leaves at $P < 0.05$ level.

Nitrogen mass balance

The mass balance of nutrients were calculated based on the assumption that the biosolids were incorporated into 10 cm soil depth, the amended soil had a soil bulk density of 1.24 g/cm^3 and nutrients in plants were analysed not after the final crop harvest but plant samples were taken at 4-5 leaf stage for canola and 5-6 tillering stage for oats. Therefore, the data were presented under these assumptions.

To examine the fate of N from land applied dewatered biosolids, various data were used. The data for the concentrations of total N in dewatered biosolids shown in Table 6.2 were used to calculate the quantity of total N loaded into the soil. In addition, data for total N in biosolids amended soil (Table 6.3) were also used to estimate the mass of total N (kg N/ha) remained in the soil at the end of the two years experiment.

For the computation of total N uptake by the two crops, the data for total N in canola and oats leaves (Table 6.7), data for canola seed and plant biomass yields from (Table 4.2 and 4.3) and oats seed and plant biomass yields from (Table 4.4 and 4.5) together with the data on concentrations of total N in canola seed (Table 4.6) were used to estimate the total N (kg N/ha) uptake by the crops at the end of each years crop harvest. Soil total N analytical data (mg/kg) were converted into kg/ha using 1.24 gm/cm^3 soil bulk density at 10 cm sampling depth.

Cropping sequence was also taken into account while calculating the total N mass balance in dewatered biosolids amended soil in canola and oat plots.

The results showed that the level of total N residue remaining in the 25, 45 and 65 t/ha dewatered biosolids amended plots for canola-oats rotation was significantly higher than the values observed in the oats-canola rotation. Conversely, N uptake by canola and oats in the oats-canola rotation plots was substantially higher than the corresponding uptake values observed in the canola-oats rotation, this difference significantly contributed for the higher values of total N recovered in the oats-canola rotation (Table 6.10).

Table 6.10 Mass balance of total nitrogen in dewatered biosolids amended canola and oats plots in the 2006 and 2007 field experiments.

Dewatered biosolids rates (t/ha)	Total-N applied (kg/ha)	Total-N residue remained in soil (kg/ha)	Plant uptake of N (kg/ha)	Total- N recovered (kg/ha)	Unaccounted N balance (kg/ha)	Unaccounted N balance in (%)
Canola-oats rotation (2006-2007)						
25	2110	868	113	981	1129	53
45	3798	1364	269	1633	2165	57
65	5486	1612	280	1892	3594	66
Oats-canola rotation (2006-2007)						
25	2110	744	127	871	1239	59
45	3798	1240	334	1574	2224	59
65	5486	1488	652	2140	3346	61

The unaccounted N balance in both cropping sequence was reasonably constant ranging between 53-66 % which indicates that more than 50 % of the total N applied into the soil was lost through various routes.

These results are similar to the observations from laboratory experiments conducted by NBRP (National Biosolids Research Program) in southern Queensland in which they showed that 30 % of the applied N was lost through denitrification during three months period of study, and suggested that high loses of N through denitrification may well be greater under wet soil condition (NBRP , 2007). Hence in this experiment, since the crops were watered twice a week using a sprinkler system, it is expected that most of the mineralized N from the dewatered biosolids treated plots was lost through denitrification and leaching of NO_3-N down the soil sampling depth. Ammonia volatilization could also be another route of N loss particularly in the dewatered biosolids.

Discussion

Total N in soil

Mineralization of nitrogen depends on mainly soil moisture and temperature and in most studies it is measured over time, rather than single end points, however in this study soil nitrogen residue was measured at the end of the crop harvest, and the findings of this study indicated that dewatered biosolids and composted biosolids applications significantly increased soil total N in all plots treated with biosolids. There were also upward trends in soil total N following dewatered biosolids and composted biosolids application rates in both years of the experiment as loading rates increased. The residual levels of total N in the fertilized control plots were not different from the unamended control plots, indicating that the plants had either used the nitrogen applied or that it was lost though volatilisation and leaching.

The observed increases were consistent with the findings of other researchers using similar biosolids types (Hartl *et al*., 2003; Tarrason *et al*., 2007). There was no significance difference between canola and oats plots in terms of the residual total N left in the soil after each year crop harvest ($p < 0.05$).

The effect of composted biosolids commulative application rates in the 2007 trial was significantly ($p < 0.05$) higher than dewatered biosolids for both canola and oat plots with respect to total N residue left in the amended soil, despite the higher concentrations of total N in dewatered biosolids. However, since nitrogen mineralization rates depends on mainly soil temperature and moisture, it was expected that a significant proportion of the total N in dewatered biosolids may have been mineralized to NO_3-N and been lost via denitrification and leaching, and this was evident from the significantly higher levels of NO_3-N leaching observed in the dewatered biosolids (65 t/ha) amended plots in the 2007 experiment (Fig 6.3).

The cropping sequence had a significant effect on the concentrations of total N residue left in the soil. In both dewatered biosolids (65 t/ha) and composted biosolids (70 t/ha) treated plots considerably less total N was accumulated for oats-canola rotation than the levels found in the canola-oats cropping sequence. This could probably be due to the biocidal chemicals released during decay of canola root residues in the soil which may act as biofumigant against soil microorganisms and thus flushing of microbial nutrients into the soil.

Kirkegaard ***et al.*** (1999) suggested this when using high rates of conventional fertilizer they found higher concentrations of accumulated N for a canola-wheat rotation than for wheat-canola cropping sequence. Alternatively, since the mineralization of organic-N in soil takes place slowly, at the end of the first year of the canola-oats rotation, a considerable N residue may have accumulated in soil. Oat crops do not use as much N as canola, and would not be as efficient to exploit the residual soil N, but in the oats-canola rotation, the canola crop would probably take more of the N residue in amended soil. Canola needs about 25 % more N, P and K than Australian Standard wheat to balance fertilizer inputs with nutrient removal in grain (Hocking ***et al***., 1999). Norton, (2003) also indicated that canola is often the first crop following pasture and benefits from the N fixed by legumes during the pasture phase.

In both dewatered biosolids and composted biosolids trials, less total N was accumulated for oats-canola rotation than for canola-oats cropping system (Table 6.4); however it was also important to note that during the 2007 experiment, the oats crops before reaching maturity were infested with stem rust and this may have an impact on the N uptake potential of the oats.

Nitrate in soil

As dewatered biosolids and composted biosolids application rates increased, upward trends in soil NO_3-N levels were observed for both crops in both years of the experiments. Dewatered biosolids had slightly greater effect in changing the NO_3-N status of the soil than composted biosolids.

Repeated application of dewatered biosolids increased the level of NO_3-N by 46 mg/kg in canola plots and by 33 mg/kg in oats plots, respectively. A repeat loading of composted biosolids increased the level of soil NO_3-N by 41 mg/kg in canola plots and by 44 mg/kg in oats plots. In dewatered biosolids amended soil a 46 mg/kg NO_3-N residue was recorded for canola- oats crop rotation whereas, a 33 mg/kg NO_3-N residue accumulated for oats-canola rotation and thus, more soil NO_3-N residues were recorded for canola-oats rotation than for oats-canola cropping sequence.

The level of NO_3-N observed in canola plots were significantly ($p < 0.05$) greater than those observed in oats plots (Fig.6.2). Compared with 2006, reapplying dewatered biosolids and composted biosolids in 2007 significantly increased the level of NO_3-N residue in the amended soil. The concentration of NO_3-N in composted biosolids treated canola plots was slightly higher than the levels recorded in the dewatered biosolids treated canola plots, and this was not unusual, since the composted biosolids had higher concentrations of NO_3-N than the dewatered biosolids. Beegle *et al*. (1994) suggested 25 mg/kg NO_3-N or greater in the background soil concentration is sufficient for maximum corn silage yield in which case no nitrogen fertilizer would be required. The soil background concentration for this experiment was 2.9 mg/kg indicating NO_3-N deficiency. Thus, in this study the highest soil NO_3-N residue recorded 57 mg/kg (71 kg N/ha) and 56 mg/kg (69 kgN/ha) in canola and oats plots amended with dewatered biosolids in the 2007 trial were below the crop NO_3-N requirement (100 and 110 kgN/ha) for canola and oats respectively.

To this end, several researchers (Rodriguez *et al*. 1996; Rodriguez *et al*. 2003; Shober *et al*., 2003) noted higher levels of NO_3-N at the higher rates of municipal solid waste compost treated plots, compared to the control plots. However, Parkinson *et al*. (1996) noted that NO_3-N concentrations in soil samples taken during the summer months were not significantly different from the unfertilized control plots suggesting cumulative NO_3-N losses during summer season. In contrast, Stamatiadis *et al*. (1999) also observed that despite initially high NO_3-N content of compost, NO_3-N levels of compost amended soil were low and similar to the control plots suggesting net loss through leaching, root uptake and possibly immobilization and denitrification processes.

In the 2007 experiment significant levels of NO_3-N residues were observed in both canola and oat plots treated with dewatered biosolids (5, 25, 45 and 65 t/ha rates) and composted biosolids (10, 30, 50 and 70 t/ha rates). This indicates that nitrogen supply from biosolids exceeds crop nitrogen requirement. Therefore, this possibly suggests that biosolids applications based on NLBAR under estimates the quantity of organic-N mineralization rates in the biosolids used in this study.

Consequently further research needs to be undertaken on mineralisation in order to more accurately match crop demand with biosolids application rate both in dewatered and composted biosilds products.

Nitrate leaching

In the 2006 experiment, the concentrations of NO_3-N determined at various soil depths of the highest dewatered application rate (65 t/ha) in the canola treatment were lower than the corresponding NO_3-N levels recorded in the oats treatment (Fig. 6.3).

The NO_3-N values recorded at the highest composted biosolids rate (70 t/ha) canola plots (0-20 cm depth) were also lower than the corresponding values recorded in oats plots treated with composted biosolids suggesting that more NO_3-N was accumulated in oats plots treated with dewatered and composted biosolids than in canola plots

The level of NO_3-N for canola lots treated with dewatered biosolids and composted biosolids were relatively similar in 2006, although more NO_3-N was observed in oats plots treated with composted biosolids than in the oats plots treated with dewatered biosolids plots (Fig.6.3).

In the 2007 experiment, NO_3-N levels in dewatered biosolids treated canola and oats plots were significantly higher than the corresponding values for canola and oats plots treated with composted biosolids, suggesting higher NO_3-N concentration was accumulated in dewatered biosolids (Fig. 6.3).

The concentration of NO_3-N accumulated in the top 20 cm of soil in the oats plots treated with dewatered biosolids was two fold greater than the levels observed in canola plots suggesting that canola with its deep rooting system may have consumed more NO_3-N from the biosolids than the oats crops.

Nitrate levels recorded at various soil depths in 2006 canola plots treated with dewatered biosolids were not different from those recorded in the 2007 canola plots ($p > 0.05$), These similarities can partly be explained due to canola's efficiency in utilizing NO_3-N in both years of the experiments, however, the level of NO_3-N in the 2007 oats plots treated with dewatered biosolids were substantially higher than those recorded in the 2006 oats plots (Fig. 6.3).

Soil NO_3-N levels in the 2007 composted biosolids treated canola and oats plots were lower when compared with the corresponding values in 2006 which was unexpected. This is in marked contrast to the dewatered biosoldis plots. The reason for the difference could be due to a decline in the mineralised nitrogen levels in the composted product used in the 2007 cropping season. This observation clearly needs further investigation and could suggest that composted biosolid may pose nitrogen management challenges compared to dewatered biosolid due to product variation and possible interactions with the soil resulting in nitrogen immobilisation.

Nitrate leaching in the first year of the field experiment was not as expected, however compared to the control plots a considerable amount of leaching was observed at 80-100 cm soil depths. Hartl *et al*. (2003) also observed increased NO_3–N leaching due to compost application in all of their treatments during the first year. However, in the second year, only the 270 t/ha treated plots resulted in higher NO_{3-}N leaching, whereas, in the third year they recorded no significant differences between any of the treatments.

Total N in plants

Over all, dewatered biosolids had a slightly greater effect on the level of TN in canola leaves than in oats. The response of oats to composted biosolids in terms of TN was not significant in either years of the experiment.

Dewatered biosolids had higher total N and NH_4- N concentrations than composted biosolids and was more bioavailable for the crops than composted biosolids (Fig.6.4).

The concentration of total N in canola and oats plants matter was significantly higher after treatment with dewatered biosolids than after treatment with composted biosolids.

In the 2007 trial, the highest total nitrogen (7.2 %) for canola leaves and in 2006 4.9 % TN for oats in were recorded at the 65 t/ha dewatered biosolids rates. According to Reuter and Robinson (1997), the critical deficiency ranges of total nitrogen in canola tissue is 6.8-6.9% and the adequate range for oats is 3.4 -5.4 %, and hence the value for both canola and oats were within the adequate ranges. The crops did not show any signs of toxicity or deficiency for nitrogen (Fig.6. 5).

The concentration of total N in canola tissue was higher than those recorded from oats tissue at the end of the 2007 experiment for both biosolids types, indicating that in this experiment canola extracted more N from the biosolids than oats (Fig.6.4).

Similarly, Hocking *et al*. (1997) also reported that compared to most other grain crops in Australia; canola has a greater requirement for nutrient inputs to achieve high yields.

Nitrates in plants

The concentrations of NO_3-N in canola and oats leaves at tillering stage increased with both biosolids application rates. The levels of NO_3-N observed in canola and oats tissue amended with dewatered biosolids was higher than the corresponding NO_3-N levels found in composted biosolids biosolids amended canola and oats crops, suggesting that the NO_3-N levels in dewatered biosolids were higher than the NO_3-N contained in composted biosolids.

The maximum NO_3-N concentrations recorded as 9154 mg/kg in canola tissue in 2007 and 7542 mg/kg in oats tissue in 2006 were well above the critical deficiency values (1620 mg/kg) for canola listed in Reuter and Robinson (1997), and therefore crops did not show any signs of toxicity symptoms for nitrate.

In general, dewatered biosolids and composted biosolids significantly changed the NO_3-N concentrations in both canola and oats tissue, with dewatered biosolids having more effect than composted biosolids.

Repeat applications of biosolids also changed the NO_3-N levels in both crops; the level of NO_3-N observed in canola tissue was significantly higher than the levels observed in oats tissue, indicating a greater uptake by canola crop.

Over all, dewatered biosolids and composted biosolids applications increased the concentrations of total N and NO_3-N both in soil and in canola and oats crops; dewatered biosolids had greater effect than composted biosolids with the exceptions of total N in soil where composted biosolids had a greater effect than composted biosolids. A considerable amount of total N and NO_3-N in the soil was accumulated; with the excess N utilized by the subsequent crop.

Biosolids application did not pose any risk from the leaching of NO_3-N down the soil profile, as most of the NO_3-N was accumulated in the top 20 cm soil depth and the findings of this study indicated that the soil NO_3-N levels leached in canola plots were low compared with the oats crop.

Since canola has deep roots and high root volume, it extracted more total N and NO_3-N than the oat crop; this was evidenced by the significantly higher concentrations of total N and NO_3-N observed in the canola leaf than in the oats leaves. Such moderate increases in tissue N may enhance crop quality and improves nutrients recycling.

For biosolids containing higher quantities of N, canola would be the preferred crop to be planted since from the findings of this result it can be suggested that canola would be used as a clean up crop in nitrate contaminated lands. In addition to this, the adoption of oats-canola cropping system would help to reduce the amount of excess nitrogen residues accumulated in soils receiving biosolids.

6.6. Phosphorus in biosolids amended soil and in plants

Several researchers (Harrison *et al*. 1994; Alves *et al*. 2006) reported significant increases in total P levels in soils at various biosolids application rates. A number of other researchers, (Stewart ***et al***. 1998; Erich ***et al***. 2002; Arbestain ***et al***. 2005; Courtney and Mullen 2007; Kidd ***et al***. 2007) also observed that high application rates of compost (100 t/ha) significantly raised plant-available phosphorus levels following manure and compost application. Phosphorus is a major plant nutrient for the production of crops, and most biosolids contain high concentrations of P; however, when biosolids are applied at a rate to satisfy the N requirement of a crop, based on nitrogen limited biosolids application rates (NLBAR), P may be excessive and accumulate in the soil creating a significant risk of off site movement through run off and may contribute to the contamination of aquifers (NBRP, 2007).

While excess soil phosphorus is not harmful to plants when biosolids are applied based on nitrogen rates (Peterson *et al.*, 1994) off-site migration to aquatic systems could be a major concern since P is the limiting nutrient in most fresh waters (Sharpley and Beegle, 1999).

To determine if this would be the case with the biosolids generated by WWSP, the total and Olsen- P concentrations in biosolids amended soil were analysed following the harvesting of canola and oats crops for two consecutive years and the results together with data validation procedures are presented below.

6.6.1. Data validation and analytical accuracy

Total - P in soil

Biosolids amended soil samples were taken each year after harvesting the crops and the total-P levels were determined using wave length dispersive X-ray fluorescence spectrometry (WD-XRF). The accuracy of total phosphorus data were validated by analysing Till 1 and Till 3 (soil standard reference materials) using WD-XRF which was externally calibrated using eight soil standard reference materials containing various elements. The percent recoveries of total-P were 120 and 116 % for Till1 and Till 3 indicating good agreement with the certified values (Table 6.11 and 6.12).

Table 6.11 Eight soil standard reference materials analyzed for total–P concentrations using XRF and expressed in (µg/g) for validating the analytical data.

Analyses	S73319	S73320	S73321	S73322	S73323	S73324	S73325	S73326
Measured	735	447	329	692	366	317	1154	773
Certified	735(28)	446 (25)	320(18)	695(28)	390(34)	303(30)	1150(39)	775(25)
Recovery %	99.9	100.1	102.7	99.6	93.8	104.6	100.4	99.8

The eight soil standard reference materials were analyzed individually to establish the calibration curve. For the measured values, the standard deviation of the calibrated P was ± 11. Values in parenthesis indicate ± SD of triplicate measurements.

Table 6.12 Concentrations of total-P for soil standard reference materials analyzed using XRF and expressed in µg/g for validating the analytical data.

Till 1	measured	Certified	Recovery %)	Till 3	measured	Certified	Recovery %)
	1112	930	120		569	490	116

Till 1 and Till3 soil standard reference materials were analyzed individually using the established calibration curves

Olsen-P

To validate the accuracy of Olsen- extractable phosphorus data, ASPAC 51 soil standard reference material was treated as a sample and analysed for phosphorus concentrations and the percent recovery was determined for each analytical run. The recovery of the certified P standard ranged between 94-108 % and was in good agreement with the certified values Table 6.13).

Moreover, Olsen extractable soil phosphorus data were also validated by spiking soil extracts with a known concentration (0.8 µg/g) of phosphorus standards. The percent recoveries of the spiked samples compared with the unspiked samples were on average 111 % (Table 6.14).

Table 6.13 Results of ASPAC 51 soil standard reference material analyzed for Olsen-P for every batch of experiments for data validation purposes.

Experiments	Measured soil-P (µg/g)	Certified-soil P (µg/g)	Recovery (%)
Exp.1[a]	14 ± 2	14.88 ± 1.01	94
Exp.2	14 ± 2	14.88 ± 1.01	94
Exp.3	14 ± 1	14.88 ± 1.01	94
Exp.4	14 ± 4	14.88 ± 1.01	94
Exp.4	16 ± 3	14.88 ± 1.01	108

Before laboratory analysis of P in biosolids amended soil soils, ASPAC 51 (Australian Soil and Plant analysis Council) soil standard reference materials were weighed and exposed to extraction procedures and analyzed using FIA for P concentrations, the measured values were compared with the certified range for accuracy purpose, whereas, for precision of the extraction procedure the coefficient of variation was calculated. All values indicate means of triplicate measurements with their standard deviation.
a: In 2007, experiment 1 and 2 refer to the biosolids amended soil samples taken from oats plots treated with dewatered and composted biosolids, whereas, Experiment 3 and 4 refers to biosolids amended soil samples taken from canola plots treated with dewatered and composted biosolids respectively

Acetic acid extractable plant phosphorus

Plant extracts were spiked with a known concentration of 0.8 and 0.08 µg/g phosphorus standards and analysed. The percent recoveries of the spiked samples compared with the unspiked samples were on average 89-103 % (Table 6.14).

Table 6.14 The recovery of soil and plant extracts spiked with various known concentrations of phosphorus standards for validating the analytical data during phosphorus analysis in soil and plant matter using flow injection analysis (mgP/L).

Soil extracts spiked with 0.8 mg/L of P-standards,2006				
	Exp.1	Exp.2	Exp.3	Exp.4
Unspiked	0.22	0.228	0.172	0.282
Spiked	1.3 ± 0.0	1.022 ± 0.026	0.9779 ± 0.0	1.123 ± 0.001
Recovery (%)[a]	135	99	106	105
Soil extracts spiked with 0.8mg/L of P-standards,2007				
Unspiked	0.2086	0.2243	0.0970	0.1567
Spiked	0.9905 ± 0.001	1.0569 ± 0.021	1.0898 ± 0.014	1.0671 ± 0.0024
Recovery (%)	97.7	104	125	113
Plants extracts spiked with 0.08mg/L of P-standards, 2006				
Unspiked	0.5819	0.5819	0.2008	0.2008
Spiked	0.6624 ± 0.015	0.6624 ± 0.015	0.285 ± 0.007	0.285 ± 0.007
Recovery (%)[b]	100.7	100.7	105	105
CV (%)	2.32	2.32	2.75	2.75
Plant extracts spiked with 0.8mg/L P-standard, 2007				
Unspiked	0.098	0.120	0.3871	0.6911
Spiked	0.508 ± 0.0005	0.525 ± 0.005	1.1839 ± 0.002	1.5015 ± 0.005
Recovery (%)	51	50.65	99	101
CV (%)	0.1	0.96	0.16	0.33

The superscript a refers to biosolids amended soil samples were extracted using Olsen's procedure, then extracts were spiked with 1 mL of the 20 mg/L P standards in 25 mL flask (0.8 mg/L) and analyzed using FIA and the percent recovery of the spiked sample related with the unspiked was calculated.

The superscript b refers to in the 2006 analysis of phosphorus in plant samples, aliquots of 0.2 g powdered plant samples were weighed and spiked with 2 mL of the 100 mg/L P standards in 100 mL flask (2 mg P/L) and 50 mL 2 % acetic acid was added and shaken for 30 minutes, filtered and diluted to 100mL with 2 % acetic acid and analyzed using 1:25 dilution factor (0.08 mg P/L) by FIA, and the percent recovery compared with the unspiked sample was calculated.

c : In the 2007 analysis of phosphorus in plant samples, aliquots of 0.2 g powdered plant samples were weighed and 50 mL of 2 % acetic acid was added and shaken for 30 minutes, filtered, then plant extracts were spiked with 1 ml of the 20 mgP/L P standard in 25 mL flask (0.8 mg/L) and analyzed , the percent recovery of the spiked sample related with the unspiked sample was calculated.

The spiked values indicate mean ± sd of duplicate measurements for each batch of experiments; however, unspiked soil and plant extracts in 2006 and 2007 were analysed individually.

6.6.2. The influence of biosolids applications on total soil-P residues

At the beginning of the experiments, dewatered biosolids and composted biosolids applications were calculated based on nitrogen limited biosolids application rates (NLBAR) and incorporated into the soil (Table 3.3. and 3.4 under section 3.2 of chapter 3). The total and Olsen- extractable P loading rates (kg P/ha) were also calculated for each biosolids types based on the P concentrations contained in the biosolids (Table 6.15 and 6.16).

A two sample t-test was conducted between the Olsen-P loading rates of dewatered biosolids and composted biosolids, and the statistical test showed that there is no significant difference between the dewatered biosolids and composted biosolids loading rate based on Olsen-P values.

The concentrations of total and Olsen-P values for soil, dewatered biosolids and composted biosolids before the start of the experiment is shown in Table 6.15, where composted biosolids had slightly higher concentration of total and Olsen-P than dewatered biosolids.

The total and Olsen-P residues in dewatered biosolids and composted biosolids amended clay loam soil were determined after each crop harvest, Figure 6.6 depicts the influence of loading various dewatered and composted biosolids on the total P status of the biosolids amended soil after two successive years of applications.

Table 6.16 presents the total and Olsen- extractable P levels loaded during the 2006 and 2007 experiments. The P levels for the 2006 unamended control plots show soil background P concentrations before planting the crops, whereas the P levels for the unamended control plots in 2007 refer to the average P concentrations calculated from the unamended control plots after first year (2006) crop harvest.

Table 6.15 Concentrations of total and extractable P in soil and biosolids used for the experiments expressed in µg/g on dry weight basis).

Analytes	Soil	Dewatered biosolids	Composted biosolids
Total-P	855 ± 3	15003 ± 4	21290 ± 566
Olsen-P	15 ± 2	691 ± 5	762 ± 7
(Olsen/XRF)*100	1.7 %	4.6 %	3.6 %

Total P and S were analyzed using WD- X-ray fluorescence spectrometry, whereas Olsen-P was determined by flow injection analyzer (FIA). Soil and biosolids samples were analyzed in triplicates.

Table 6.16 Dewatered biosolids and composted biosolids applications and their corresponding total and Olsen-P levels in 2006 and 2007 field experiments.

Biosolids types	2006		2007		
Dewatered application rates (t/ha)	Total-P kg/ ha	Olsen-P kg/ ha	Dewatered application rates (t/ha)	Total-P kg/ ha	Olsen-P kg/ ha
0	0	0	0	0	0
5	75	3.5	5	75	3.5
25	375	17.3	25	375	17.3
45	675	31.1	45	675	31.1
65	975	44.8	65	975	44.8
Composted application rates (t/ha)			Composted application rates (t/ha)		
10	223	7.6	10	223	7.6
30	669	22.8	30	669	22.8
50	1115	38.0	50	1115	38.0
70	1560	53.2	70	1560	53.2

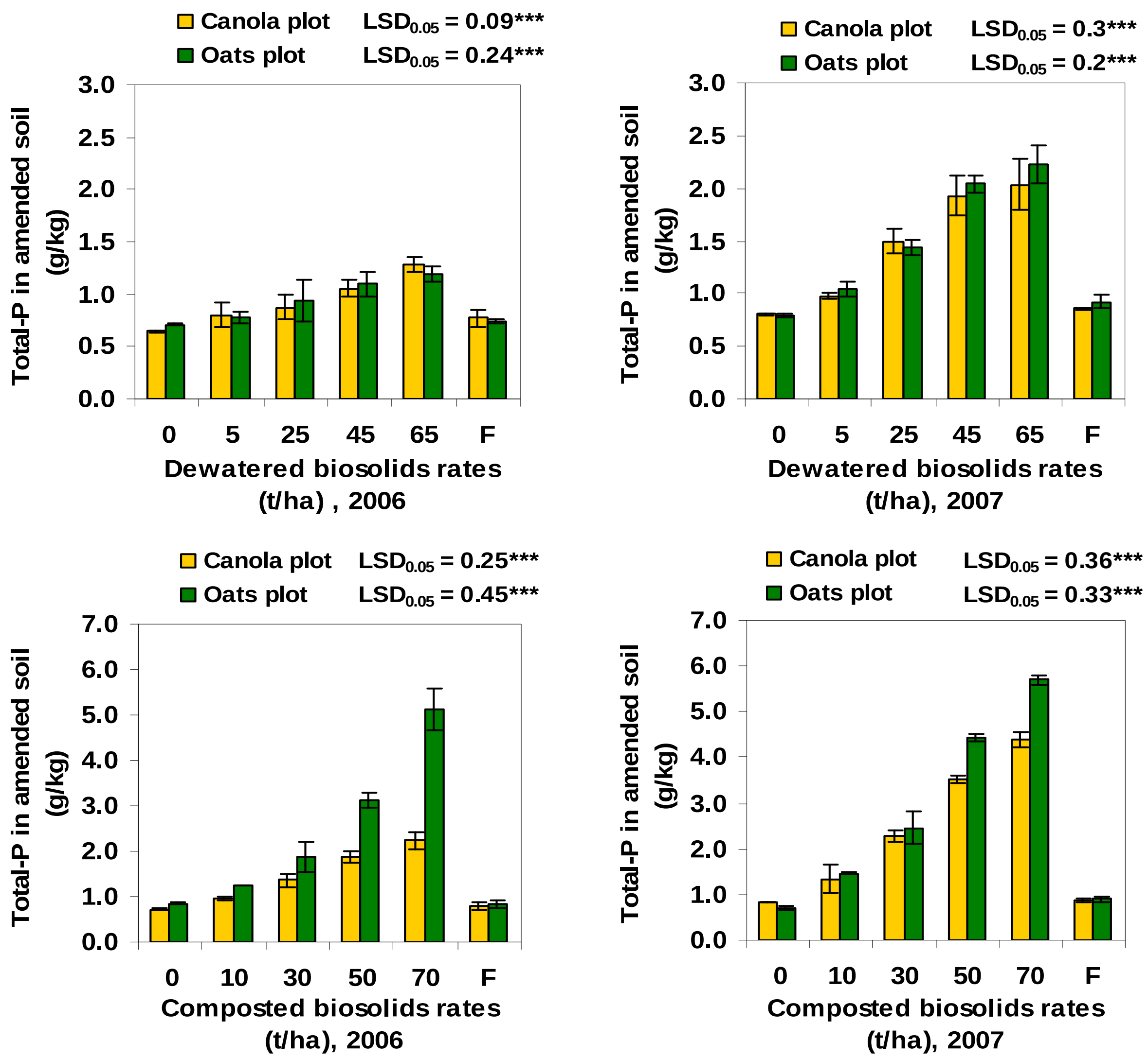


Figure 6.6 The effect of dewatered and composted biosolids application rates on the level of total phosphorus in amended soil in 2006 and 2007 field experiments. The error bars represent standard deviations of triplicate measurements. LSD $_{0.05}$ values refers to the least significant difference (t-test) between the mean values of total-P in biosolids amended soil at 5 % probability, whereas *** refers to significant treatment effects in ANOVA (F-test) at p < 0.001 level.

Year 1

Total phosphorus levels in the highest dewatered biosolids amended plot (65 t/ha) in 2006 experiment doubled, changing from 0.65 to 1.3 g/kg for canola and from 0.71 to 1.2 g/kg for oats plots compared with the control plots,. In the composted biosolids experiment, when total phosphorus levels in the control plot were compared with the highest composted biosolids application (70 t/ha), it had increased from 0.72 to 2.2 g/kg (three fold) and from 0.84 to 5.2 g/kg (six fold) for canola and oats plots respectively.

There was no significance difference in total P levels between canola and oats plots treated with dewatered biosolids; however, in composted biosolids treated oats plots the level of total-P was higher by 2.8 g/kg as would be expected than the level observed in canola plots (Fig.6.6 and Table 6.18).

The level of total P observed in canola plots treated with composted biosolids was higher by 0.87 g/kg than the total P levels found in canola treated with dewatered biosolids plots. An even greater difference was observed in the case of oats plots, where the total P levels in the plots treated with composted biosolids was higher by 3.8 g/kg than in the oat plots treated with dewatered biosolids, indicating more residual total P in plots treated with composted biosolids than with dewatered biosolids treatments (Fig.6.6 and Table 6.18).

Year 2

In the 2007 trial, when compared with the control plot, a three fold increase in total P concentrations was observed at the highest (65t/ha) dewatered biosolids loading rate which on average increased from 0.8 to 2.04 g/kg for canola and from 0.79 to 2.2 g/kg for oats. Again, there was much more residual total P left in plots treated with composted biosolids than those treated with dewatered biosolids (Fig.6.6 and Table 6.18).

When the responses of canola and oats to biosolids phosphorus were compared, there was no significant difference between the total P levels recorded in canola and oats plots treated with dewatered biosolids. However, in composted biosolids treated plots, a 1.43 g/kg more total P was noted in oats plots than in canola plots treated with composted biosolids, indicating that composted biosolids treated oats plots had more total P concentrations than the corresponding total P levels in canola plots.

When, the influence of the two biosolids types were compared, a significantly (2.3 g/kg) greater amount of total-P in canola plots treated with composted biosolids was observed than the corresponding total P levels recorded in dewatered biosolids treated canola plots. Similarly, the level of total P recorded in oats plots treated with composted biosolids was higher by 3.6 g/kg than the level recorded in oats plots treated with dewatered biosolids plots (Fig.6.6 and Table 6.18).

Repeated application of dewatered biosolids changed the concentration of total P in canola plots from 0.64 to 1.2 g/kg, whereas, total-P levels in oats plots treated with dewatered biosolids changed from 0.5 to 1.43 g/kg which indicated more total P residue left in oats plots treated with dewatered biosolids.

Reapplying composted biosolids changed the concentration of total P in canola plots from 1.5 to 3.6 g/kg. Similarly, the level of total P in composted biosolids treated oats plots changed from 4.3 to 4.98 g/kg.

6.6.3. Olsen-P

Year 1

The level of Olsen-extractable soil phosphorus in the 65 t/ha dewatered biosolids amended soil in 2006 trial changed significantly ($p < 0.05$) from 24 to 52 mg/kg in canola and from 20 to 43 mg/kg in oats plots respectively, whereas in the 70 t/ha composted biosolids treated plots Olsen-P changed from 21 to 69 and from 14 to 99 mg/kg in canola and oats plots respectively.

Comparing the influence of crops on Olsen-extractable phosphorus in biosolids amended soil, the amount of phosphorus observed at the highest application rates of canola and oats plots treated with dewatered biosolids were similar and no significant difference in Olsen-P levels were noted between canola and oats plots treated with composted biosolids (Table 6.17 and 6.18).

When the two biosolids types were compared, there was no significant difference in Olsen-P between dewatered biosolids treated canola and composted biosolids treated canola plots; however, when the P concentrations recorded in the unamended control plots was subtracted from P concentrations obtained at the highest biosolids application rates, the net concentrations of Olsen-P (84.6 mg/kg) observed at the highest (70 t/ha) composted biosolids treated oats plots was significantly ($p < 0.05$) higher than the level recorded (23 mg/kg) in oats plots treated with dewatered biosolids (Table 6.17 and 6.18).

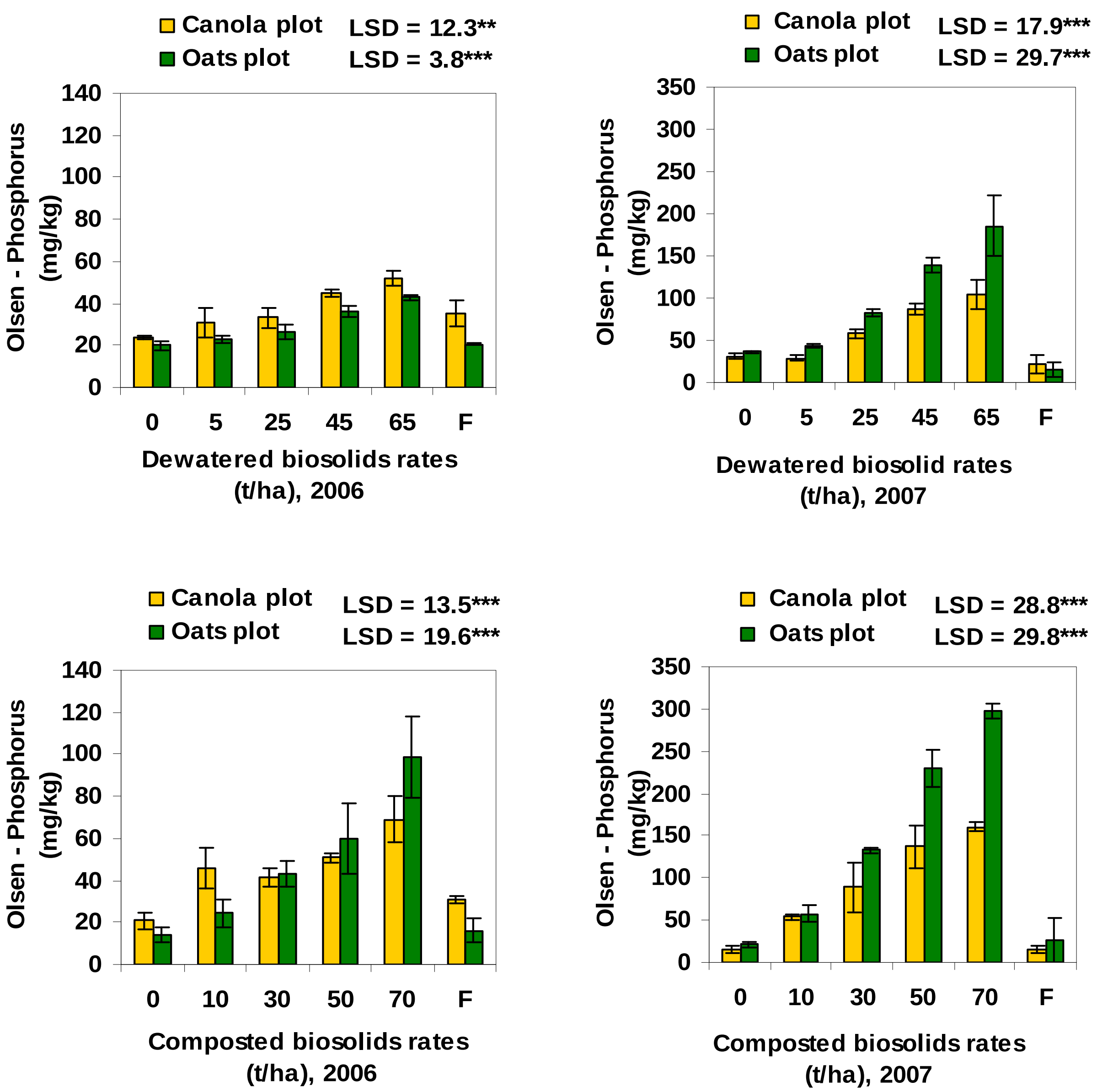


Figure 6.7 The effect of dewatered biosolids and composted biosolids application rates on the level of Olsen- extractable phosphorus in amended soil in 2006 and 2007 field experiments.
The error bars represent standard deviations of triplicate measurements.
LSD $_{0.05}$ values refers to the least significant difference (t-test) between the mean values of total-P in biosolids amended soil at 5 % probability, whereas *** refers to significant treatment effects in ANOVA (F-test) at p < 0.001 level.

Table 6.17. The effect of dewatered biosolids and composted biosolids applications on total and Olsen- extractable soil-P in canola and oats plots during 2006-2007 field experiments, (mg/kg on dry weight basis)

Biosolids application rates (t/ha)	Canola plots, 2006		Oats plot, 2006		Canola plots , 2007		Oats plot, 2007	
	Total-P	Olsen-P	Total-P	Olsen-P	Total-P	Olsen-P	Total-P	Olsen-P
Effect of dewatered biosolids applications on total and Olsen-P in amended soil								
0	647±12	24±1	708±9	20±2	804±14	21±3	793±14	15±1
5	797±117	31±7	780±54	23±2	973±28	29±3	1042±80	43±2
25	876±110	33±5	942±202	27±3	1497±119	58±6	1440±71	82±4
45	1054±81	45±2	1094±116	36±3	1930±196	87±6	2047±80	139±8
65	1290±74	52±3	1193±74	43±1	2035±237	104±17	2224±179	186±35
Fertilized	770±82	35±4	741±17	21±1	853±16	31±11	921±65	37±9
LSD $_{0.05}$	93***	12.27**	235***	3.84***	310***	17.95***	161***	29.72***
Effect of Composted biosolids applications on total and Olsen-P in amended soil								
0	719±24	21±4	841±26	14±4	831±11	15±4	714±34	21±3
10	947±44	46±10	1262±8	24±6	1338±299	54±29	1456±16	58±3
30	1362±138	42±4	1876±330	43±6	2291±127	89±25	2450±354	133±22
50	1884±132	51±2	3131±158	60±17	3527±84	137±6	4432±87	229±10
70	2232±196	69±11	5126±446	99±19	4389±185	161±4	5699±94	297±26
Fertilized	780±89	31±2	840±72	16±5	862±29	16±3	910±62	26±10
LSD $_{0.05}$	254***	13.48***	480***	19.58***	356***	28.82***	327***	29.79***

Values indicate means ± sd of triplicate measurements, whereas, ** and *** refers to significant treatment effects in ANOVA (F-test) at $p < 0.01$ and $p < 0.001$ levels respectively.

Table 6.18 Relative percentages changes in Olsen extractable and XRF total soil P concentrations at the highest dewatered (65 t/ha) and composted (70 t/ha) biosolids amended in canola and oats plots

P in dewatered biosolids treated canola plots				P in dewatered biosolids treated oats plots		
P	P levels namended plot	P levels at 65 t/ha amended plot	Increases (in %)	P levels unamended plot	P levels at 65 t/ha amended plot	Increases (in %)
Olsen-P						
Yr 1	24	52	117	20	43	115
Yr 2	21	104	395	15	186	1140
Total-P						
Yr 1	647	1290	99	708	1193	69
Yr 2	804	2035	153	793	2224	180
P in composted biosolids treated canola plots				P in composted biosolids treated oats plots		
P	P levels unamended plot	P levels at 70 t/ha amended plot	Increases (in %)	P levels in unamended plot	P levels at 70 t/ha amended plot	Increases (in %)
Olsen-P						
Yr 1	21	69	229	14	99	607
Yr 2	15	161	973	21	297	1314
Total-P						
Yr 1	719	2232	210	841	5126	510
Yr 2	831	4389	428	714	5699	698

The increases in Olsen and XRF determined soil P concentrations were expressed in percent for the P concentrations obtained from the highest dewatered (65 t/ha) and composted (70 t/ha) biosolids application plots compared to the P concentrations recorded from the unamended control plots, whereas P levels were expressed in mg/kg.

Year 2

In the 2007 trial, repeat application of dewatered biosolids increased the Olsen-P levels changing from 21 to 104 mg/kg in canola plots and from 15 to 186 mg/kg in oats plots respectively. However, Olsen-P levels in composted biosolids treated plots changed from 15 to 161 mg/kg in canola plots and from 21 to 297 mg/kg in oats plots respectively ($p < 0.001$). When the levels of net soil P residue (less the control plots) remained in canola and oats plots were compared, the level of Olsen-P residues (171 mg/kg) found in oats plots treated with dewatered biosolids were significantly greater than those recorded (83 mg/kg) in canola plots. Similarly, Olsen-P levels recorded (276 mg/kg) in oats plots treated with composted biosolids were significantly greater than those found (146 mg/kg) in canola plots ($p < 0.01$) (Table 6.17 and 6.18).

When the effect of the two biosolids types on Olsen-P levels were compared, 62 mg/kg more Olsen-P was found in canola plots treated with composted biosolids than the level recorded in dewatered biosolids treated canola plots. Similarly, 105.5 mg/kg more Olsen-P was also found in oats plots treated with composted biosolids than the levels recorded in dewatered biosolids treated oats plots ($p < 0.01$).

The application of dewatered biosolids in canola plots resulted in an increase in Olsen-P values by 28 and 83 mg/kg in 2006 and 2007 respectively ($p < 0.05$), while applying dewatered biosolids in oats plots increased the levels of Olsen-P by 23 and 171 mg/kg in 2006 and 2007 respectively ($p<0.01$) (Table 6.17).

Composted biosolids application in canola plots increased the level of Olsen-P by 48 and 146 mg/kg in 2006 and 2007 respectively, whereas Olsen-P in oats plots increased by 85 and 276 mg/kg in 2006 and 2007 trials respectively ($p < 0.01$) (Table 6.17).

Effect of biosolids applications on the ratio of Olsen-P to XRF total P

In the 2006 dewatered and composted biosolids treated canola and oats plots, the ratio of Olsen-P to XRF total levels did not show any trend in response to biosolids applications, however in the 2007 experiments, there was a significant increasing trend following dewatered and composted biosolids applications (Fig.6.8).

The change in the ratio of Olsen to XRF total P levels in the 2007 dewatered biosolids treated plots were substantially higher than the levels observed in composted biosolids amended canola and oats plots.

As discussed in chapter 5 section 5.5.1, dewatered biosolids applications had significant effect in lowering the pH of the soil in both years of the experiment. Such decrease in soil pH would possibly increase the solubility of organic P in the dewatered biosolids amended soil and thus increasing the ratio of Olsen-P to XRF total P levels following dewatered biosolids application.

As described in chapter 5 section 5.5.2 composted biosolids application increased the soil pH in the 2006 experiment, but in 2007, the effect of composted biosolids in increasing the soil pH might have been offset by the process of organic-N mineralization (nitrifications) which generally releases H^+ into the soil system. Thus, in the 2007 composted biosolids treated plots, the ratio of Olsen-P to XRF total P levels significantly increased (Fig.6.8).

In 2007, the ratio of Olsen-P to XRF total P levels in oats plots treated with dewatered and composted biosolids was significantly higher than the levels observed in canola plots; this suggests that canola took up more biosolids P than the oat crops.

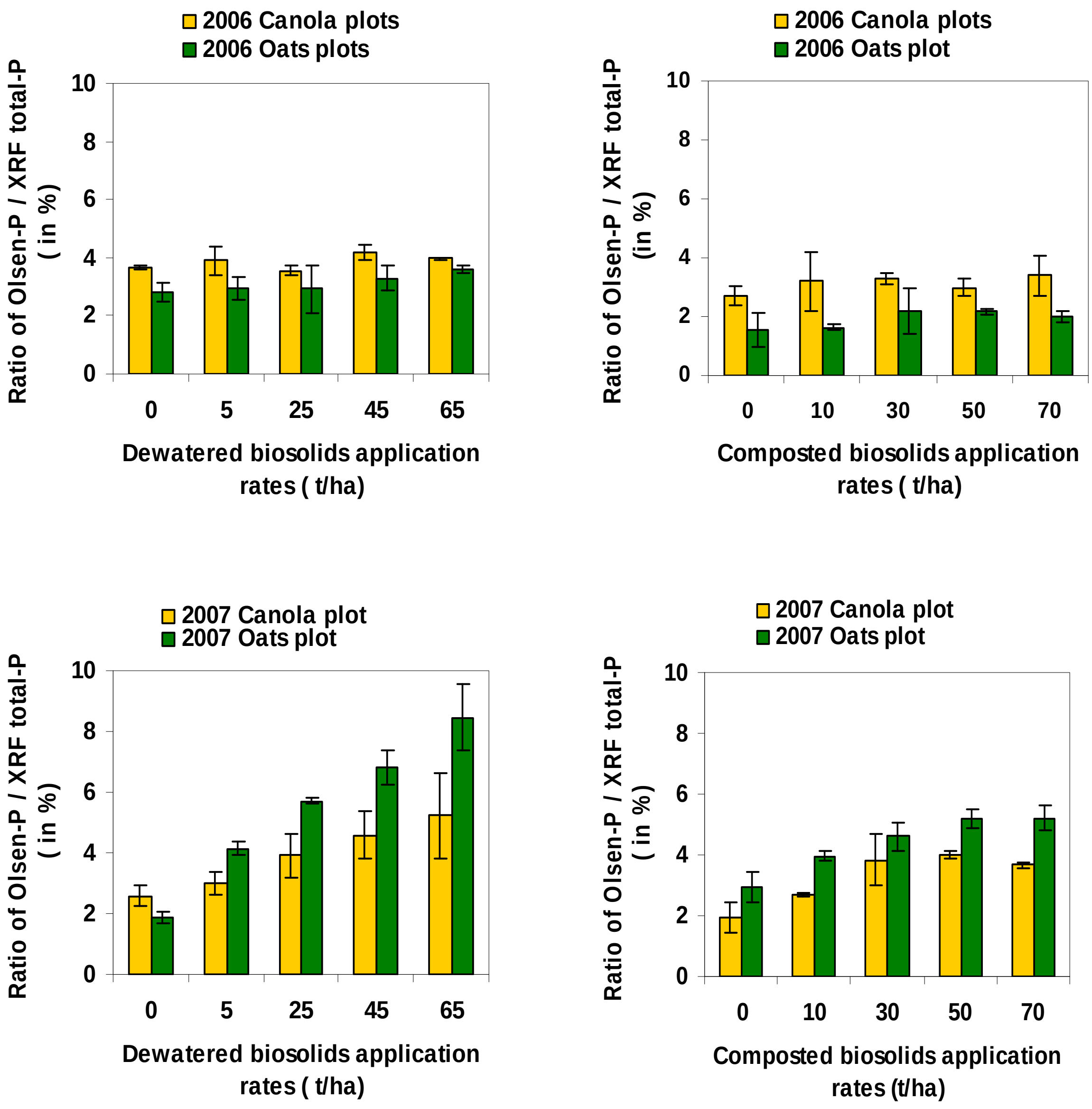


Figure 6.8 The influence of dewatered and composted biosolids application rates on the ratio of Olsen-P to XRF total P levels in canola and oats plots, in the 2006 and 2007 experiments.

6.6.4. Effect of cropping sequence on Olsen-P residue

As shown in Table 7.19, crop rotation had significant effect on total N and Olsen-P residues accumulated in the biosolids amended soil after the end the growing season.

Table 6.19 The effect of cropping sequence on the level of Olsen-P accumulated in dewatered and composted biosolids amended canola and oats plots, in 2006 and 2007 experiments(in mg/kg)

Cropping sequence in dewatered biosolids treated plots							
Analytes	Yr1 canola	Yr2 oats	Yr 2-Yr 1	Analytes	Yr1 oats	Yr2 canola	Yr 2-Yr 1
Olsen - P	30	171	143	Olsen - P	23	83	60
Cropping sequence in composted biosolids treated plots							
Analytes	Yr1 canola	Yr2 oats	Yr 2-Yr 1	Analytes	Yr1 oats	Yr2 canola	Yr 2-Yr 1
Olsen - P	48	276	229	Olsen - P	85	145	61

Dewatered and composted biosolids amended soil samples were taken after each year crop harvest and Olsen - P was determined using flow injection analysis. The symbol Δ indicates the difference between year 1 and year 2 concentrations of Olsen - P residue remained at the end of the two years period.

In the canola-oats cropping sequence, 83 mg/kg more Olsen-P residue was accumulated than in the oats-canola rotations for dewatered biosolids amended plots, similarly, in composted biosolids amended plots, in the canola-oats cropping sequence 168 mg/kg more Olsen-P residue was left than the oats-canola crop rotations.

6.6.5. Impact of biosolids application on Olsen-P

Regression analysis of the two years Olsen-P data showed that a one tonne application of dewatered biosolids in 2006 raised the Olsen-P values by 0.22 and 0.35 mg/kg of the amended soil for canola and oats plots respectively. In 2007, after repeated application of dewatered biosolids, a one tonne dewatered biosolids application further increased the Olsen-P levels by 0.66 and 1.27 mg/kg for canola and oats plots respectively.

In the 2006 composted biosolids treated plots, a one tonne application of composted biosolids increased the Olsen-P levels by 0.54 and 1.15 mg/kg for canola and oats plots. Similarly, after repeated application of composted biosolids in 2007, a one tonne application of composted biosolids increased the Olsen-P values by 1.03 and 2.01 mg/kg for canola and oats plots (Table 6.20).

Table 6.20 The impact of biosolids loading rates on Olsen-P residues accumulated in soil in 2006 - 2007 field experiment (mg/kg).

Biosolids types	Intercept	Regression Coefficients	R^2
Dewatered biosolids, 2006			
Canola plots	34***	0.22ns	0.18
Oats plots	20***	0.35***	0.94
Dewatered biosolids, 2007			
Canola plots	23***	0.66***	0.94
Oats plots	22**	1.27***	0.95
Composted biosolids, 2006			
Canola plots	28***	0.54***	0.70
Oats plots	11*	1.15***	0.87
Composted biosolids, 2007			
Canola plots	25**	1.03***	0.91
Oats plots	19*	2.01***	0.98

The superscripts *,**,*** and ns refers to significance of the intercept and regression coefficients (t-test) at $P < 0.05$, $P < 0.01$, $P < 0.001$ and not significant respectively. The intercept and regression coefficient values for the 2007 experiment refer to the impacts of dewatered and composted biosolids on Olsen-P levels after two years successive application.

6.6.6. Acetic acid extractable plant-P

Concentrations of acetic acid extractable orthophosphate expressed from petioles and leaves have been used for rapid field tests for the diagnosis of the phosphorus status of plants (Ulrich and Hills, 1990). In addition, acetic acid extractable phosphorus in the leaf blades of grapevines at flowering stage provided a sensitive measure of the phosphorus status of the vines for prediction of their berry weight and yield and pruning weight (Skinner *et al*., 1987). Likewise, Brenan and Bolland (2005) indicated that the concentration of phosphorus in canola grain was double that of wheat confirming that canola used all sources of phosphorus more effectively than wheat to produce dried shoot and grain.

Barbarick *et al*. (2004) applied composted biosolids at various rates and observed that soil and plant tissue P concentrations of stream bank wheatgrass increased with increasing application rates of biosolids which significantly enhanced forage quality and improve nutrient cycling. Kidd *et al*. (2007) observed that P uptake was significantly greater in plants growing on amended soils; however, tissue P content in maize did not differ greatly between plants grown on control or amended soils. Under South Western Australian soil condition Pritchard (2005) also reported increased P uptake by wheat and lupin following biosolids application in a field experiment.

To diagnose P deficiency levels in canola and oats leaf due to dewatered biosolids and composted biosolids applications and to further examine the difference in canola and oats in terms of uptake of P, the concentrations of P in canola and oats leaf was extracted using 2 % acetic acid and analysed using flow injection analysis. The results are presented below:

Year 1

Plant phosphorus concentrations in canola and oats leaves in 2006 dewatered biosolids treated plots were significantly higher than the control plots ($p < 0.05$ and $p < 0.001$) changing on average from 4.7 to 6.5 g/kg for canola and from 2.0 to 4.4 g/kg for oats respectively. The highest plant phosphorus level in canola and oat was observed at the 65 t/ha dewatered biosolids application rate. However, compared with the control plots, no significant increases in plant phosphorus levels were observed in the 2006 composted biosolids treated canola and oats leaf suggesting dewatered biosolids had greater impact in changing the phosphorus status of canola and oats leaf than composted biosolids (Table 6.21).

Table 6.21 The effect of dewatered biosolids and composted biosolids applications on acetic acid extractable phosphorus in canola and oats leaf in the 2006 and 2007 field experiments

Dewatered biosolids rates (t/ha)	2006		2007	
	P in canola leaf (g/kg)	P in oats leaf (g/kg)	P in canola leaf (g/kg)	P in oats leaf (g/kg)
0	4.7 ± 0.4)	2.0 ± 0.6	1.9 ± 0.2	0.13 ± 0.07
5	5.2 ± 0.7	2.1 ± 0.6	2.8 ± 0.3	0.30 ± 0.3
25	5.6 ± 0.7	3.9 ± 0.5	3.6 ± 0.2	0.47 ± 0.6
45	5.9 ± 0.1	4.0 ± 0.8	4.3 ± 0.5	0.70 ± 0.8
65	6.5 ± 0.6	4.40 ± 0.04	5.1 ± 0.2	0.80 ± 0.2
Fertilized	5.1 ± 0.3	1.6 ± 0.1	1.7 ± 0.2	0.37 ± 0.3
$LSD_{0.05}$	1.04*	0.91***	0.40***	0.30***
Composted biosolids rates (t/ha)				
0	4.5 ± 0.6	2.5 ± 0.2	3.1 ± 0.2	0.2 ± 0.1
10	4.6 ± 0.53	3.2 ± 0.7	4.2 ± 0.4	0.4 ± 0.3
30	5.7 ± 0.83	3.3 ± 0.9	4.4 ± 0.1	0.8 ± 0.2
50	5.5 ± 0.4	4.3 ± 0.4	3.8 ± 0.1	0.8 ± 0.1
70	5.2 ± 0.8	4.4 ± 1.2	4.2 ± 1.3	1.2 ± 0.4
Fertilized	4.60 ± 0.04	2.1 ± 0.3	3.1 ± 0.2	0.4 ± 0.2
$LSD_{0.05}$	ns	ns	1.0*	0.52**

The superscripts ***, **, * and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively, figures in parenthesis indicate standard deviations of triplicate measurements.

Year 2

In the 2007 repeat dewatered biosolids trial, plant phosphorus concentrations significantly ($p < 0.001$) changed from 1.9 to 5.1 for canola and from 0.13 to 0.8 g/kg for oats respectively.
Similarly, significant plant phosphorus concentrations were also observed in the 2007 composted biosolids treated canola and oat plots which changed on average from 3.1 to 4.4 for canola and from 0.2 to 1.2 g/kg for oats respectively (Table 6.21).
Plant phosphorus concentrations observed in canola and oats plant matter in the 2007 composted biosolids treatment was significantly lower than the 2006 recordings.

Phosphorus mass balance

As in the case of N, a similar procedure was followed for the computation of P mass balance in dewatered biosolids amended canola and oats plots. The concentrations of total P values in dewatered biosolids shown in Table 6.15 was used to calculate the quantity of total-P loading rates from biosolids. Soil and plant P data presented in Table 6.17 and 6.21 together with canola seed and plant biomass (Table 4.2 and 4.3) and oats seed and plant biomass (Table 4.4 and 4.5) were used respectively. Data on canola seed P concentrations (Table 4.6) was also employed in the calculation of P residue in soil and P taken up the plants after two years successive applications of dewatered biosolids.
Whether cropping sequence had any effect on the quantity of soil P residue remaining in the amended soil was also examined. The higher dewatered biosolids (25, 45 and 65 t/ha) amended canola and oats plots were chosen for the calculations of P balance, during the computation, total-P concentrations measured in the soil background concentration were subtracted from the corresponding dewatered biosolids amended plots to differentiate changes due to dewatered biosolids applications only. Similarly, plant P concentrations, seed and plant biomass yield values of the unamended control plots were subtracted from the corresponding values obtained from dewatered biosolids treated plots.

Table 6.22 Mass balance of total phosphorus in dewatered biosolids amended canola and oats plots in the 2006 and 2007 experiments.

Dewatered biosolids rates (t/ha)	Total-P applied (kg/ha)	Total-P residue in soil (kg/ha)	Plant uptake of P (kg/ha)	Total-P recovered (kg/ha)	Unaccounted P balance (kg/ha)	Unaccounted P balance in (%)
Canola- oats rotation						
25	750	637	14	651	99	13
45	1350	1334	27	1361	-11	-0.8
65	1950	1476	35	1511	439	22
Oats- canola rotation						
25	750	649	24	673	77	10
45	1350	1090	51	1141	209	15
65	1950	1169	78	1247	703	36

For the computation of P mass balance in dewatered biosolids amended soil, the soil total P background concentration before the start of the experiment (855 mg/kg) was taken from Table 7. 15 and subtracted from the total P residuals remaining at the 25, 45 and 65 t/ha application rates at the end of the two years experiment (Table 7.17) . Using 1.24 gm/cm3 soil bulk density at 10 cm soil depth, the mg/kg values were converted into kg/ha. For the calculations of P uptake (kg/ha), concentrations of plant P data presented in Table 7.21 was taken and multiplied by the total seed and plant biomass yields of canola and oats (Table 4.4 and 4.5), the P concentrations in canola seed from Table 4.6 was also considered while calculating the P uptake by canola and oats in dewatered biosolids amended soil.

The quantity of total-P recovered in the canola-oats cropping sequence was slightly higher than the values recorded in the oats-canola rotation. Results of the computation also showed that total soil P residues accumulated in the canola-oats crop sequence was significantly higher than the residues found in the oats-canola rotation. Similar to the findings of N mass balance, the observed P uptake in the oats-canola rotation was considerably higher than the levels observed in the canola-oats rotation (Table 6.22).

The unaccounted P balance ranged between -0.8-22 % and 10-36 % for the canola-oat and for oats-canola rotations respectively. The negative value (-0.8) observed for the unaccounted P balance at the 45 t/ha treated plots was not expected, however it is expected that it would be due to the highly heterogeneous nature of the biosolids while sampling and analysis for total P concentrations in the biosolids. As dewatered biosolids application rates increased, the percent unaccounted P balance also increased. Though P levels below the 10 cm soil depth was not measured, it is expected that this would be due to the effect of the sprinkler watering system which tends to dissolve the water soluble P pool particularly at the higher P applied plots and enhanced P loss through leaching down the 10 cm soil sampling depth.

Repeat applications of dewatered biosolids, the results of the total-P mass balance indicated that the unaccounted total-P values were not high, to this end the choice of crops and cropping sequence had an effect both on soil P residue remained in soil and on the P uptake by the two crops.

6.7. Sulfur in biosolids amended soil

Sulphur (S) is not a very abundant element; however, it can come from various origins such as action of surface water, ground water, or sea water, the biological contribution of different animals and plants. Agricultural, industrial and domestic activities also contribute to soil improvement in S compounds, particularly organic amendments, compost, fertilizer and pesticides. With the exception of swampy histisols, gypseous soil and acid sulphated soil, the S content of most soil is very low.

The occurrence and stability of the different S derivatives largely depends on the redox potential and soil pH (Sparks, 1996). Several organic and inorganic fractions of S occur in soil and in organic matter (William, 1987). Total S values range between 3 to over 10,000 µg/g with a mean value around 430 µg/g. Measurement of soil total S might be helpful to assess the size of S pool in soil (Rayment and Higginson, 1992).

Recently in Australia, much attention has been given to the role of S in canola production, since the high S content of canola oil suggests that S uptake is higher than for other crops. Greater responses of canola compared with wheat to S applications have been recorded, particularly when heavy nitrogen applications have resulted in high yields (Hocking *et al*., 1996). Canola has a greater requirement for S than several other crops, including cereals and grain legumes (Holmes 1980; Colton and Sykes, 1992).

Mc Bride *et al*. (2004a: 2004b) reported that organic-rich sludge products (dewatered, pelletized, composted) increased soil extractable S consistent with a relatively high concentration of S in anaerobically-digested sewage sludge. Similarly, Lavado *et al*. (2005) in field experiments using digested and undigested biosolids applied on maize crops, reported that the S levels of the amended soil was significantly increased due to biosolids applications.

6.7.1. Data validation and analytical accuracy

For XRF analysis of total-S in soil/biosolids and biosolids amended soils, external calibration curve for total-S was established by analysing eight soil standard reference materials (NCS DC 73319, NCS DC 73320, NCS DC 73321, NCS DC 73322, NCS DC 73323, NCSDC 73324 NCS DC 73325 NCS DC 73326). Table 7.23 shows the measured and certified values of these standard reference materials.

To validate the established calibration curve, Till1 and Till3 soil standard reference materials obtained from Canada were treated as samples and analysed for total-S values. The percentage recoveries of each of the metals compared with the certified values are shown in Table 6.24

The recoveries of the eight soil standard reference materials analysed for total-S values were all in good agreement with the certified values (ranging from 66.7 % to 128.2 %) with exceptions of the reference material S73320 where the recovery (66.7 %) was very low. Recoveries for till 1 and till 3 soil standard reference material for total-S values were also within the certified ranges (Table 6.23 and Table 6.24).

Table 6.23 Eight soil standard reference materials analyzed for total–S concentrations using XRF to establish a calibration curve (values expressed in µg S/g)

Analyses	S73319	S73320	S73321	S73322	S73323	S73324	S73325	S73326
Measured	286.2	193.8	113.5	166.2	378.5	240	230.8	116.3
Certified	277.1	175.5	75.7	213	340.8	261.7	240	141.5
Recovery %	96.82	90.56	66.70	128.16	90.04	109.04	103.99	121.67

Table 6.24 Concentrations of total-S for soil standard reference materials till 1 and till 3 analyzed using XRF and expressed in % for validating the analytical data.

Till 1	measured	Certified	Till 3	measured	Certified
Total-S levels	0.025	< 0.05	Total-S levels	0.013	< 0.05

Till 1 and Till3 soil standard reference materials were treated as samples and analyzed individually using the established calibration curve

Year 1

In the 2006 biosolids field experiment, the application of dewatered biosolids changed the concentration of S from 533 to 836 mg/kg in canola plots and from 532 to 673 mg/kg in oats plots respectively, whereas, composted biosolids loading increased the S status of the soil from 576 to 754 mg/kg in canola plots and from 665 to 999 mg/kg in oats plots respectively (Fig. 6.9 and Appendix H Table H-2).

When the two biosolids types were compared, the concentration of soil S found in the 2006 oats plots treated with composted biosolids (331 mg/kg) was significantly higher than the level (142 mg/kg) recorded in dewatered biosolids treated oats plots, however S levels found in the 2006 canola plots treated with dewatered biosolids was not significantly different from those found in composted biosolids treated canola plots.

The level of S recorded between canola and oats plots treated with dewatered and composted biosolids was not significantly different.

Year 2

In the 2007 field experiment, further reapplication of dewatered biosolids increased the concentrations of S from 248 to 728 mg/kg in canola plots and from 288 to 713 mg/kg in oats plots respectively. In composted biosolids treated canola and oats plots the level of S increased from 258 to 927 mg/kg for canola and from 394 to 790 mg/kg for oats plots respectively (Fig. 6.9).

The level of S found in 2007 canola plots treated with composted biosolids (668 mg/kg) was slightly higher than the levels recorded (480 mg/kg) in canola plots treated with dewatered biosolids, however no significant differences were noted between S levels recorded in oats plots treated with dewatered and composted biosolids.

There was no significant difference in the level of soil S between canola and oats plots treated with dewatered biosolids. Sulphur levels recorded in 2007 canola plots treated with dewatered biosolids were slightly higher but not statistically significant than the S levels recorded in the 2006 canola plots treated with dewatered biosolids plots indicating that reapplying dewatered biosolids in 2007 to some extent increased the S status of the soil. There was a highly significant difference between S levels recorded in 2006 and in 2007 canola plots treated with composted biosolids. Likewise, S levels recorded in the 2007 oats plots treated with (65 t/ha) dewatered biosolids (425 mg/kg) were significantly higher than the S levels (142 mg/kg) recorded in the 2006 dewatered biosolids treated oats plots; however, the 2006, S concentrations recorded in oats plots treated with composted biosolids were not significantly different from the 2007 S levels recorded in oats plots treated with composted biosolids (Appendix G Table G - 2).

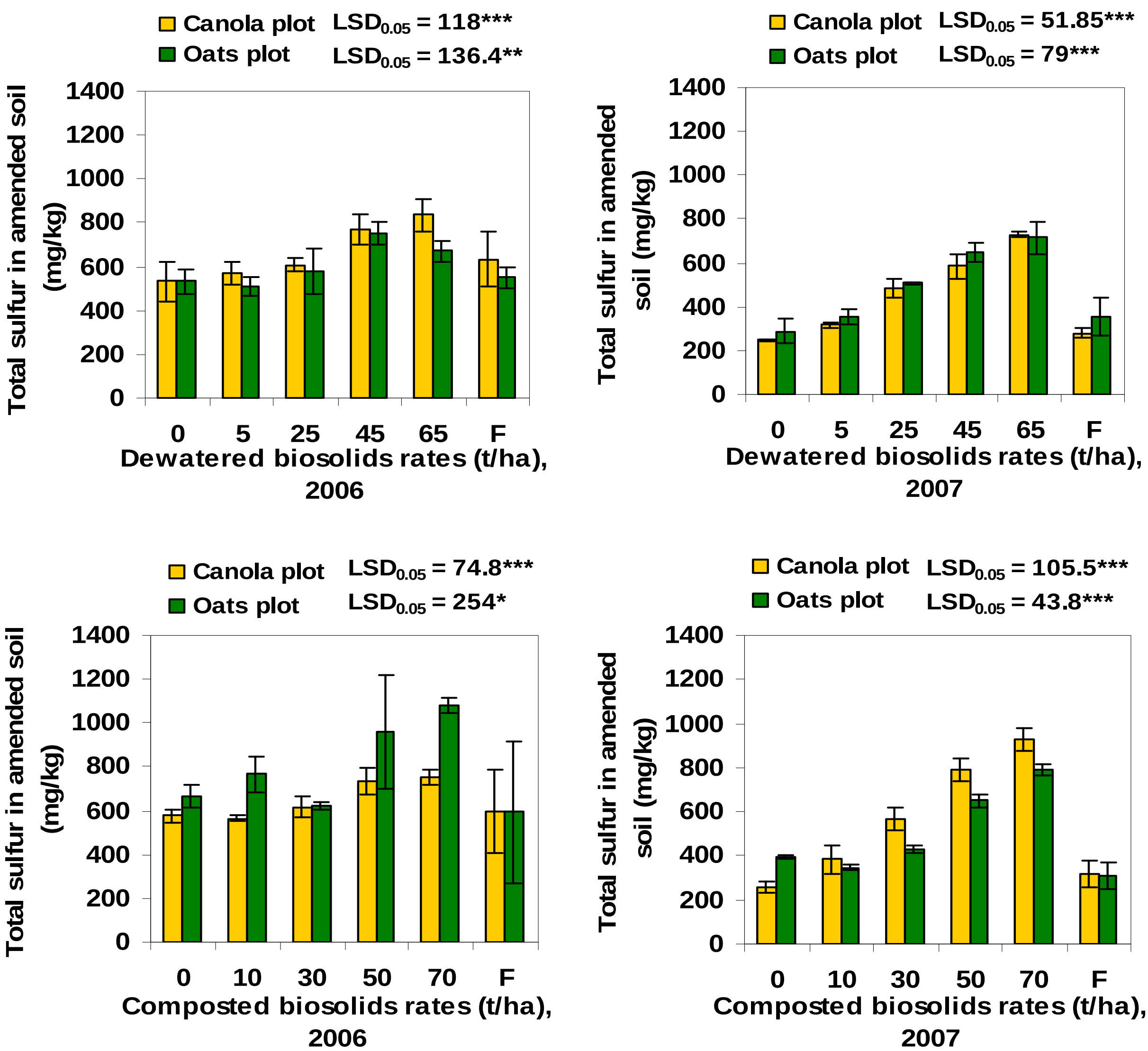


Figure 6.9 The effect of dewatered and composted biosolids loading rates on the level of total sulfur in amended soil in 2006 and 2007 field experiments. The superscripts *, ** and *** refer to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively whereas, the error bars indicate standard deviation of triplicate measurements.

Discussion

Total and Olsen-P in soil

Upward linear trends in total soil P levels were noted with both biosolids types in both years of the experiments (Fig.6.6). As expected, dewatered biosolids and composted biosolids applications significantly increased the level of total- P in the amended soil compared with the control plots in both years, however, during the two years period of the experiments, the observed increases in plots treated with composted biosolids were increased on average by 4 and 7 fold for canola and oat plots respectively compared with dewatered biosolids treated canola and oat plots.

Similarly, dewatered biosolids and composted biosolids applications significantly changed the Olsen-P status of the soil. The effect of composted biosolids in changing the phosphorus status of the soil was significantly higher than dewatered biosolids treated plots in both years for both crops. The highest soil P concentrations were observed at the 65 and 70 t/ha maximum biosolids loading rates.

Both biosolids application rates significantly increased the level of Olsen-P showing an increasing trend and exceeded the agronomic requirement (usually 20 kg P/ha) for the crops. Table 7.17 shows the influence of the two different sources of biosolids on phosphorus availability under oats and canola after the first and second year crop harvest. The dewatered biosolids rate (5 t/ha) and composted biosolids rate (5 t/ha) would possibly supply sufficient Olsen-P for canola and oats to grow without P deficiency.

The study clearly showed that, in both dewatered biosolids and composted biosolids treated plots a considerably higher Olsen-P residue was accumulated for canola-oats rotation than the levels found in the oats-canola cropping system.

It could be suggested that the substantially higher soil Olsen-P residues left in the canola-oats cropping sequence would be due to the effect of organic acids and phosphatase enzymes which may have been released by canola roots could possibly tend to break the organic matter and dissolve the soluble P, therefore raising the plant available P pool. It may also be due to poor P uptake by oat crop in the 2007 experiment

Obviously, in both dewatered biosolids and composted biosolids experiments less Olsen-P was accumulated for oats-canola rotation than for canola-oats cropping system (Table 6.19).

The impact of composted biosolids in raising the Olsen-P values was substantially higher than dewatered biosolids, and this was evidenced by the higher regression coefficients observed in composted biosolids treated plots (Table 6.20).

Likewise, when canola and oats crops were compared, higher Olsen-P levels were observed in oats plots than in canola plots for both biosolids types and in both years of the experiments. This was further confirmed by the higher regression coefficients observed in oats plots than in canola plots which indirectly suggest that canola's P uptake was significantly higher than the oat crops.

Despite luxuriant supply, no phosphorus toxicity symptoms were observed on any specimen of either crop.

Extractable plant-P in canola and oats

Results of plant leaf analysis for phosphorus showed that dewatered biosolids applications increased phosphorus concentrations in canola and oats leaves; however, the concentration of phosphorus in canola leaf was significantly higher than that of oat leaves which would indicate that canola extracts more phosphorus from the soil than oats. Canola due to its deep root and greater root volume and its ability to release organic acids and solubilise soil P, has the advantage in exploiting and absorbing more P than oats (Table 6.21).

Likewise, Marschner *et al*. (2007) in a commercial fertilizer experiment reported that despite similar concentrations of available P in the soil; total shoot P content was greater in two canola genotypes than the wheat genotypes. They concluded that canola genotypes had a greater dry weight and took up more P than the wheat genotypes. They also noted that the canola genotypes had a greater root length compared to the wheat genotypes which could lead to a greater soil volume exploited, and a greater capacity to respond to P addition.

Canolas have also been shown to increase P solubility by release of organic acid anions (Hoffland, 1992; Hoffland *et al*., 1989). Phosphorus solubility can be increased by excretion of organic acid anions into the rhizosphere and/or change the rhizosphere pH (Gerke and Meyer, 1995; Imas *et al*., 1997). Phosphatases released by plant roots or soil microorganisms can mineralize organic P (Tarafdar and Claassen, 1988), thus increasing P availability.

According to Reuter *et al*. (1997) the adequate range of plant tissue phosphorus values are 3-4.8 g/kg for canola at 120 days after sowing and 3-5 g/kg for oats at mid and late tillering stage (Appendix I Table I-1 and I-2). The highest canola tissue phosphorus concentration recorded (6.5 g/kg) in 2006 slightly exceeded the adequate range, whereas the highest oats tissue phosphorus concentrations recorded in 2006 was 4.4 g/kg and this value was in the adequate range.

Total S in soil

The S status of the amended soil was increased due to the higher concentration of both the dewatered biosolids and composted biosolids used for the experiments. As dewatered biosolids and composted biosolids application rates increased, a considerable upward trend in the sulphur status of the amended soil was noted.

Although dewatered biosolids had significantly higher S concentration (11380 mg/kg) than composted biosolids (5264 mg/kg) Table 7.11, the impact of the two biosolids in raising the S status of the soil was relatively similar.

In the 2006 control plots, higher S levels were observed than in the 2007 control plots, this was because gypsum ($CaSO_4.2H_2O$) at a rate of 3 t/ha was applied uniformly to all the plots to prevent dispersion and crusting of the soil before sowing the canola and oat seeds.

Regarding the effect of crop rotation on the level of S residue left in dewatered biosolids amended soil; it was observed that a 135 mg/kg more sulphur was accumulated for oats-canola rotation than for canola-oats cropping system. Likewise, for composted biosolids treated canola and oats, 1164 mg/kg more sulphur was observed for oats-canola rotation than canola-oats cropping. Results of this study showed that dewatered biosolids and composted biosolids additions on crop land would help farmers to decrease or be a substitute for inorganic S fertilisers.

6.8. Relationships between soil and plant nutrients in biosolids amended soil

Plant uptake of nutrients particularly N and P is largely influenced by the nutrient status of soil, and hence determining the relationship between nutrients in soil and plants helps to evaluate and make environmentally sound recommendations for biosolids application. Pearson correlation coefficients were estimated to examine the relationship among soil and plant nutrients following various biosolids amendment rates. The results are presented below.

6.8.1. Correlations between total-N and NO_3-N in soil and plant tissue

Total nitrogen and nitrate nitrogen in plants measured at 4-5 leaf stage for canola and at 5-6 tillering stage for oats usually helps as a diagnostic test for the nutrient status of the crop, however to examine whether a relationship exists between soil total and nitrate nitrogen residue left at the end of the cropping season were tested for Pearson correlation with total nitrogen and nitrate nitrogen in plant shoots measured at 4-5 leaf stage for canola and at 5-6 tillering stage for oats.

Dewatered biosolids amended plots

Pearson’s correlation analysis of soil and plant nutrient data in the 2006 trial showed that there were significant correlations between TN in soil and TN in canola and oats tissues for both crops grown on dewatered biosolids amended soil (r = 0.58* and r = 0.73**, n = 15).

The correlation between total nitrogen in soil and NO_3-N in canola and oats tissue were significant (r = 0.71** and r = 0.62*, n = 15). The correlations between NO_3-N in soil and NO_3-N in canola and oats tissues were r = 0.49 and r = 0.83** respectively.

For the 2007 dewatered biosolids treated canola and oat plots, the correlations between TN in soil and total nitrogen in canola and oats tissue was again significant (r = 0.90** and r = 0.77**, n = 15) for canola and oats respectively. The correlations between total nitrogen in soil and NO_3-N in canola and oats tissue were also highly significant (r = 0.95** and r = 0.91**). Highly significant positive correlations were also noted between NO_3-N in soil and NO_3-N in canola and oats tissue (r = 0.90** and r = 0.92**) (Table 6.23).

Composted biosolids amended plots

In the 2006 composted biosolids trial, the correlations between total nitrogen in soil and total nitrogen and NO_3-N in canola and oats tissue were poorly correlated and not significant.

As in the case of dewatered biosolids treated plots, in the 2007 composted biosolids trial, total nitrogen in soil and in canola and oats tissue were positively correlated (r = 0.79** and r = 0.61*), whereas only in canola plots that total nitrogen in soil and NO_3-N in canola tissue were positively correlated (r = 0.93**), however, there were no significant correlations between NO_3-N in soil and in canola and oats tissue (Table 6.24).

6.8.2. Correlations between total and extractable phosphorus in soil and in plant tissue

Dewatered biosolids amended plots

Table 6.23 depicts the correlations between total and extractable phosphorus in soil and in the plant under different regimes of biosolids applications in 2006. Total phosphorus in soil and extractable phosphorus in canola and oats tissue were r = 0.63* and r = 0.79** , respectively. The correlations between Olsen-P and extractable-P in canola and oats tissue were (r = 0.31 and r = 0.73**), whereas the correlations between total phosphorus in soil and Olsen-P were r = 0.51* and 0.82** for canola and oats plots, respectively (Table 6.23).

The correlation between total phosphorus in soil and extractable-P in canola and oats tissues were highly significant (r = 0.93** and r = 0.91**), whereas the correlations between Olsen-P and extractable phosphorus concentrations in canola and oats tissue were r = 0.92** and r = 0.84** respectively.

Similarly, correlations between total phosphorus and Olsen-P in canola and oats plots amended with dewatered biosolids were highly significant (r = 0.90** and r = 0.94**). Compared with the 2006 recordings, in 2007 highly significant correlations between total phosphorus in soil and extractable phosphorus in canola and oats tissues were observed (Table 6.23).

Composted biosolids amended plots

In the 2006 trial, the correlations between total phosphorus in soil and extractable phosphorus in oats tissue was significant (r = 0.66**), but total soil phosphorus in soil and extractable phosphorus in canola tissue was not significantly correlated. Similarly, Olsen-P and extractable phosphorus in canola and oats tissue were weakly correlated (r = 0.31 and r = 0.53*). However, total phosphorus and Olsen-P were strongly correlated (r = 0.80** and 95**) for canola and oats plot respectively (Table 6.24).
In the 2007 trial, the correlations between total phosphorus in soil and extractable phosphorus in canola tissue was not significant, but total phosphorus in soil was significantly correlated with extractable phosphorus in oats tissue (r = 0.77**). But, correlations between Olsen-P and extractable phosphorus in canola tissue was not significant, nevertheless Olsen-P was significantly correlated with extractable phosphorus in oats tissue (r = 0.78**). Strong positive correlations between total phosphorus in soil and Olsen-P were observed in canola and oats plots (r = 0.95** and 0.98**) respectively (Table 6.24).

Most of the total P in biosolids is in inorganic forms and the exact mineralization rates have not been determined, except in Western Australia where 100 % of the total inorganic P is assumed to be in available forms, In this study, as expected total P and available P are strongly correlated and also with P uptake as shown in Table 6.23 and 6.24.

Table 6.23 Pearson's correlation coefficient matrix between nutrients in soil and plant in dewatered biosolids amended canola and oats plots in 2006 and 2007 field experiments.

Nutrients	TN_s	TN_{pt}	NO_3-Ns	NO_3-N_{pt}	P_T	Olsen-P	P-ext $_{pt}$
Canola plots amended with dewatered biosolids in 2006 experiment							
TNs	1						
TNpt	0.58*	1					
NO_3-Ns	0.69**	0.80**	1				
NO_3-Npt	0.71**	0.68**	0.49	1			
P_T	0.75**	0.80**	0.80**	0.65**	1		
Olsen-P	0.10	0.52*	0.30	0.41	0.51*	1	
P-ext pt	0.51*	0.75**	0.66**	0.56*	0.63*	0.32	1
Canola plots amended with dewatered biosolids in 2007 experiment							
TNs	1						
TNpt	0.90**	1					
NO_3-Ns	0.96**	0.90**	1				
NO_3-Npt	0.95**	0.82**	0.90**	1			
PT	0.96**	0.89**	0.95**	0.93**	1		
Olsen-P	0.96**	0.86**	0.96**	0.93**	0.90**	1	
P-ext pt	0.95**	0.88**	0.92**	0.92**	0.93**	0.92**	1
Oats plots amended with dewatered biosolids in 2006 experiment							
TNs	1						
TNpt	0.73**	1					
NO_3-Ns	0.72**	0.85**	1				
NO_3-Npt	0.62*	0.87**	0.83**	1			
PT	0.69**	0.81**	0.80**	0.67**	1		
Olsen-P	0.83**	0.89**	0.93**	0.81**	0.82**	1	
P-ext pt	0.87**	0.68**	0.67**	0.56*	0.79**	0.73**	1
Oats plots amended with dewatered biosolids in 2007 experiment							
TNs	1						
TNpt	0.77**	1					
NO_3-Ns	0.88**	0.84**	1				
NO_3-Npt	0.91**	0.86**	0.92**	1			
P_T	0.97**	0.80**	0.89**	0.93**	1		
Olsen-P	0.94**	0.77**	0.90**	0.87**	0.94**	1	
P-ext pt	0.93**	0.72**	0.84**	0.84**	0.91**	0.84**	1

The superscripts * and ** refers to significant Pearson's correlations (t-test) at $p < 0.05$ and $p < 0.01$ levels, whereas the subscripts s refers to soil, pt for plant, T for total and ext for extractable respectively

Table 6.24 Pearson's correlation coefficient matrix between nutrients in soil and plant in composted biosolids amended canola and oats plots in 2006 and 2007 field experiments.

Nutrients	TN_s	TNpt	NO_3-Ns	NO_3-Npt	P_T	Olsen-P	P-ext pt
Canola plots amended with composted biosolids in 2006 experiment							
TNs	1						
TNpt	0.16	1					
NO_3-Ns	0.84**	0.005	1				
NO_3-Npt	0.53*	-0.08	0.22	1			
P_T	0.92**	0.36	0.84**	0.44	1		
Olsen-P	0.87**	0.03	0.87**	0.36	0.80**	1	
P-ext pt	0.47	-0.03	0.21	0.75**	0.45	0.31	1
Canola plots amended with composted biosolids in 2007 experiment							
TNs	1						
TNpt	0.79**	1					
NO_3-Ns	0.88**	0.59*	1				
NO_3-Npt	0.93**	0.74**	0.94**	1			
P_T	0.96**	0.81**	0.87**	0.95**	1		
Olsen-P	0.95**	0.83**	0.83**	0.89**	0.95**	1	
P-ext pt	0.26	0.19	0.39	0.30	0.24	0.33	1
Oats plots amended with composted biosolids in 2006 experiment							
TNs	1						
TNpt	0.61*	1					
NO_3-Ns	0.64*	0.25	1				
P_T	0.92**	0.60*	0.74**	-	1		
Olsen-P	0.88**	0.50*	0.76**	-	0.95**	1	
P-ext pt	0.67**	0.61*	0.45	-	0.66**	0.53*	1
Oats plots amended with composted biosolids in 2007 experiment							
TNs	1						
TNpt	0.76**	1					
NO_3-Ns	0.73**	0.35	1				
NO_3-Npt	0.83**	0.77**	0.67**	1			
P_T	0.97**	0.72**	0.69**	0.86**	1		
Olsen-P	0.97**	0.76**	0.72**	0.91**	0.98**	1	
P-ext pt	0.76**	0.73**	0.48	0.86**	0.77**	0.78**	1

The superscripts * and ** refers to significant Pearson's correlations (t-test) at $p < 0.05$ and $p < 0.01$ levels, whereas the subscripts s refers to soil, pt for plant, T for total and ext for extractable respectively

6.9. Conclusion

Application of dewatered and composted biosolids significantly increased the total and extractable nitrogen and phosphorus levels in soil and plant tissues. It also increased the sulfur status of the amended soil.

Results of the study indicated that biosolids can be used as sources of nitrogen and phosphorus for enhancing crop production. However, a considerable amount of Olsen-extractable P remained in the biosolids amended soil after crop harvest and this exceeded the agronomic requirements of both crops.

Phosphorus could be exported through run off or the accumulated phosphorus could be used for producing a subsequent crop. Clearly the available soil phosphorus could also support weed growth which would reduce runoff availability but compromise crop quality.

In this study, a significantly greater amount of Olsen-P and total-N accumulated for canola-oats cropping sequence than for oats-canola crop rotation for dewatered biosolids and composted biosolids treated plots which could probably be either due to the break down of biosolids organic P by organic acids and phosphatase enzymes released by canola roots in the canola-oats rotation sequence or it could be due to poor oat growth in the 2007 experiment.

The observed NO_3-N concentration leaching down in the 20 cm soil depth of the canola plots was significantly lower than the oats plots confirming canola's greater capacity to absorb NO_3-N from biosolids amended soil.

The threat for ground water contamination due to leaching of NO_3-N down the soil profile was negligible and did not pose any problem, since most of the NO_3-N was accumulated on the top 20 cm soil depth.

Growers may benefit from accumulated biosolids nitrogen and phosphorus through adopting a rotational cropping of canola after oat in the subsequent year in order to utilize the biosolids nitrogen and employ the phosphorus accumulated in the receiving soil.

Total nitrogen and extractable phosphorus in canola plant matter was significantly higher than in oat leaves indicating that canola extracted more nitrogen and phosphorus from amended soil than oats. Due to its high nutrient (N, P and S) requirements, canola can thus be used as a clean up crop particularly in contaminated lands.

The correlations between total nitrogen in soil and total nitrogen and NO_3-N in canola and oats shoot were significant. Likewise, significant positive correlations were also noted between total phosphorus in soil and extractable phosphorus in canola and oats which suggests that biosolids were the sources of nutrients for the crops. In addition to this, the soil test values for N, NO_3-N and Olsen-P could be used to predict the plant available and uptake of these nutrients in biosolids amended clay loam soil.

Moreover, as there is an interest in biofuel production using the harvested canola crops it will be important to adopt a systems approach to the application of the two crops selected for trial to determine if there are economic benefits in having higher biosolids loadings on smaller areas of land supporting canola for biofuel production compared with lower loadings supporting oats for livestock fodder.

It is already well recognized that monocropping of either crop may not be sustainable and a rotation will be essential. Opportunities for breeding crops specifically for biosolids utilization and biofuel processing should be explored as this experiment has demonstrated that both crop selection and type of biosolids had an impact upon nutrient uptake and dry matter production.

To this end, for the long term efficient utilization of nutrients from biosolids as well as to increase the adoption of using biosolids by crop growers, an investigation into the survival of pathogens of health concern in the biosolids amended soil needs to be further examined. The next chapter presents an evaluation of the survival of pathogens in biosolids amended soil after each year's successive application of biosolids.

7

CHAPTER 7. PATHOGENS IN BIOSOLIDS AMENDED SOIL

Introduction

Recycling of biosolids back to agricultural land offers an important outlet for sewage sludge and other sludges types (USEPA, 1999; Zhou, 2002; Li & Wu, 2003), however, there is a concern regarding pathogens when biosolids and sewage sludge are applied on to crop lands (Deportes *et al*., 1995; Lewis & Gattie, 2002; Sahlstrom, 2003).

Pathogens entering the soil during the application of sewage sludge to land can stay viable in the soil for two months or more (USEPA, 1994). However, most pathogens do not survive in soils or on plants for very long periods of time (Rudolfs *et al*., 1950, 1951; Lance, 1977; Akin *et al*., 1978; Golueke, 1983; Sorber and Moore, 1986). Bacteria survival in the soil depends on many factors including temperature (Martin *et al*., 1990; Mawdsley *et al*., 1995; Garrec *et al*., 2003), moisture (Ward *et al*., 1981; Russ and Yanko, 1981), pH, soil composition and the presence of competitive or antagonistic organisms (Hussong *et al*., 1985; Sidhu *et al*., 2001; Pietronave *et al*., 2004) all of which affect pathogen survival as discussed in the literature review section 2.6.1

Sewage sludges are treated in a variety of ways designed to reduce the number of pathogens in the resulting biosolids. The common treatment methods include digestion, alkali-stabilization, composting, irradiation and pasteurizations (USEPA 1994; Ho *et al*., 2000). Factors such as predation, oxygen, soil type and texture also influence pathogen inactivation.

Pepper *et al*. (1993) conducted laboratory and field experiments using total coliforms, fecal coliforms, and fecal streptococci organisms and reported that moisture, temperature, and texture of soil affected the survival of these indicator organisms. Survival of organisms was enhanced by increasing soil moisture and clay content and diminished by higher soil temperature. However, under field conditions, when rainfall increases soil moisture, regrowth of indicator organisms can occur.

This was reported by Gibbs *et al*. (1997) who showed that though concentrations of *fecal coliform*, *fecal streptococci* and *Salmonella* concentrations decreased through an extended hot, dry summer and were not detected, repopulation occurred after precipitation.

Pathogens in dewatered biosolids material applied to land will not likely leach out and move through the surface soil to groundwater and surface application, including tilling into the upper 15 cm greatly reduces the survival and movement of bacteria (Reddy *et al*., 1981).

The survival rate of pathogens on plants is very short since desiccation and ultraviolet light are the most important factors in destroying pathogens on plants. Thus, the risk for humans consuming foods where biosolids are land applied is even low since most of the biosolids are incorporated into the soil and do not come in contact with edible food crops. The risk to humans from pathogens in biosolids that are applied to non-edible crops, forestry, and fruit trees is essentially nil (Epstein, 2003).

Thus, this chapter presents results for the survival of pathogens in biosolids amended soil after each year's successive application of dewatered biosolids and composted biosolids in 2006 and 2007 field experiments. Biosolids amended soil samples were taken and analysed for *E. coli*, *Clostridium perfringens* and *salmonella spp* after each year crop harvest in the 2006 and 2007 field experiments.

7.1. Pathogen survival in biosolids amended soil after crop harvest

After harvesting the first and second biosolids field experiments in 2006 and 2007, twenty soil cores of dewatered biosolids amended soil samples from the canola plots at 0-10 cm soil depth were taken from each of the four dewatered biosolids (treatments) and from control and conventionally fertilized plots. Composted biosolids amended soil samples from the highest application rate (70 t/ha plots) were also taken.

The samples were immediately taken to the Australian Laboratory Services (ALS Consulchem) and analysed as per Australian Standards, (AS5013.11.1, 2004) for survival of pathogens: *E.coli*, *Clostridium perfringens* and *salmonella species*. The results are shown in Table 7.1.

Table 7.1 Survival of pathogens in biosolids and biosolids amended soil after 12 months of biosolids application in 2006 and 2007 at Surbiton Park.

Dewatered biosolids rates	Mean *E.coli*/g		Mean *C.perfringens* cfu/g		Mean *Salmonella* spp/25g	
	2006	2007	2006	2007	2006	2007
Control	<0.3	<0.3	<10 (0)	<10	nd	nd
Fertilized plots	<0.3	<0.3	126 (203)	<10	nd	nd
5	<0.3	<0.3	430 (118)	<10	nd	nd
25	<0.3	<0.3	423 (113)	370	nd	nd
45	<0.3	<0.3	333 (117)	530	nd	nd
65	<0.3	0.48	397 (354)	700	nd	nd
LSD $_{0.05}$	ns	ns	ns	ns	-	-
Compost(70t/ha)	<0.3	<0.3	16.7	<10	nd	nd
Dewatered biosolids(06)	2.3	-	4300	-	<3	<3
Dewatered biosolids(07)	-	43	-	590	<3	<3
Composted biosolids (07)	<0.3	<0.3	70	-	nd	nd
Infective dose	10^4 /g		10^6 cfu/g		10^2/25g	
EPA, Victoria limit	<100/g sample		-		<1/5g sample	

Figures in parenthesis indicate standard deviations of triplicate measurements, whereas cfu refers to colony forming units, and nd stands for not detected.

Results of the 2006 field trial showed that concentrations of *E.coli* in dewatered and composted biosolids amended soil were below the detection limit. The concentrations of *E.coli* in dewatered biosolids samples in the 2007 plot experiment was 43 *E.coli*/g of sample which is 16 times higher than the dewatered biosolids used in 2006 plot experiment; the likely explanation for this is because the 2006 dewatered biosolids had been left in a stockpile for a long time while the dewatered biosolids used in 2007 were fresh.

The concentrations of *E.coli* in dewatered biosolids amended soil samples after second crop harvest in 2007 were all below 0.5 *E.coli* per gram of biosolids amended soil samples for all biosolids application rates, which means *E.coli* levels in all biosolids amended treatment plots were not statistically different from the control plots.

The concentrations of *Clostridium perfringens* varied slightly across the various dewatered biosolids application rates showing no consistent trend in any of the dewatered biosolids treated plots.

Even though the soil background concentrations of *Clostridium perfringens* was very low, the levels of *Clostridium perfringens* observed in the 2006 dewatered biosolids (4300 cfu/g) was within the range of most soil background concentrations (10^3-10^4) (Curtis and Lawley, 2003).

C*lostridium perfringens* species can naturally be found in most soils as background concentrations. The presence of spores of these organisms in most cases is used as surrogate indicators of protozoan parasites, and enteric viruses; there would be a possibility that the bacteria might have multiplied in the stockpiles, however *Clostridium perfringens* can be found in small numbers (up to 10^3 cfu/g) in the faeces of healthy individuals and large numbers of cells (at least 10^6 cfu /g of food) have to be ingested to cause food poisoning (Samuel *et al*, 1991).

Thus, the relatively low concentrations of *Clostridium perfringens* species observed in dewatered biosolids amended soil are unlikely to induce any health risk.

Salmonella species in both dewatered and composted biosolids treated plots were not detected.

The concentration of *E.coli* and *salmonella* species in dewatered biosolids amended soil were very low compared with the EPA's regulatory limits for safety and health hazards of biosolids used for land application.

7.2. Conclusion

High soil temperatures during the summer season (2006 and 2007) and low pH and desiccation are likely to have had a negative impact on the survival of any pathogens remaining in the soil after application of the biosolids.

The Victorian EPA guidelines for grade T1 "unrestricted" use of biosolids requires an *E.coli* measure of < 100 per gram of dry biosolids and < 1 *Salmonella* per 5 g of sample. The results of this study showed that after two years of repeated land applications of dewatered biosolids, the level of pathogens surviving in biosolids amended soils was very low and hence the biosolids comply with the bacterial requirements so far tested for unrestricted agricultural use.

The previous chapters showed that biosolids contain valuable nutrients and hence are important environmental resource. The next chapter explains the economic value of this resource.

8

CHAPTER 8. AN ASSESSMENT OF THE ECONOMIC VALUE OF BIOSOLIDS

Introduction

This chapter presents a comparative assessment of the experimental valuation techniques employed to value the environmental goods (biosolids) as a factor of input in the production process. It describes two intertwined areas of the experimental technique in the valuation of biosolids.

Two scenarios are presented; the first scenario explains the use of biosolids as factor of input in the production process to produce a marketable output (canola seed). The second scenario uses laboratory experimental data to estimate the price of biosolids from their nutrient value (N, P, and K) perspective.

Results of the two scenarios are compared and discussed from the producer's profit maximization view point.

It also calculates the optimum quantity of canola oil produced per hectare of land at the optimum dewatered biosolids and composted biosolids application rates, and the energy value of canola oil that can be obtained from a hectare of land.

This chapter was included in order to emphasize the broader implication and recommendation that would emerge from the investigation and tie up the research work.

8.1. Scenario 1. The Productivity Change Method

"Environmental goods and services are the chemical, physical and biological relationships between organisms and their surrounding processes, attributes or the products that relate to the balance of an ecosystem which include the provision of wildlife habitat, cycling of carbon, nitrogen, phosphorus, sulfur, water or the trapping of nutrients and the basis of sustenance and prosperity to the society" (Mishra, 2000).

The ways in which the environment can act as an input in the production are potentially quite varied Environmental factors influence output by changing the productivity of inputs, by altering output that has been produced, or by reducing the effective supply of inputs. As long as the price of inputs is not affected, all of these effects can be modelled by including an environmental input in the production function.

Not all valuation studies are of the same quality or provide the same level of information about the analysis undertaken and possible sources of bias in estimates (Morrison, 2001).

However, the production function approach incorporates a dose response relationship between the environmental input (biosolids) and the outputs produced (canola seed) and effect on production techniques. The effect on production technique estimates the value of a change in the condition of the environment from an associated change in the level of production or from a change in productivity which can work in both directions (Robinson, 2001).

In valuing the environment resource as a consumption good, several researchers have dealt with pricing the environment as an input in the production process. The theoretical aspects of this valuation technique are described in Freeman, (1993), Freeman and Harrington, (1990), Maler, (1992), and Point, (1994).

8.1.1. Conceptual Framework

In valuing an environmental good, the productivity change method assumes that a direct or an indirect relationship exists between the marketed good or service and the non-marketed environmental good or service. Depending on the type of relationship, different techniques can be used to infer the economic value of the non-priced environmental good or service.

In this method, both dewatered and composted biosolids were assumed to be the environmental goods and considered as an input in the production of a marketed good (canola seed yields). Changes in environmental attributes lead to changes in the output of the marketed good. The value of the change in the environmental attribute is therefore estimated as the change in the value of production (Markandya *et al.*, 2000).

In the productivity change method, the conceptual model assumes that the environmental improvement will allow the producers to expand output and that production costs are constant (prices and quantities of inputs and factors do not change). Furthermore, producers face a perfectly elastic (horizontal) demand curve. The implication behind the assumption is that the price of output remains unchanged and the benefits generated by the environmental improvement will only accrue to crop growers.

Under these conditions, a downward shift in the supply function will be unlikely to influence the market equilibrium and the benefit generated by the change in the environmental good will accrue to the producers only. Benefits generated by the environmental improvement can be measured by observing only the changes in the producer surplus. The basic assumption of this method is that a dose-response relationship exists between the non–marketed environmental attribute (biosolids as fertilizer substitute) and the production of the marketed good (canola seed). The environmental benefit (value) is the additional benefit in terms of additional canola seed quantity generated by the increase in the usage of biosolids as a fertilizer substitute to grow the crop.

The Production Function

A production function portrays an input-output relationship. It describes the rate at which resources are transformed into products, and can be expressed as an algebraic equation:

$Y = f(X, X_m)$ ……. ………………………………………………………….Equation1

Where X is the non-marketed environmental attribute (biosolids) and X_m is a vector of marketed input that takes part in the production of Y (crop seed yield).

The production function is vital for computation of profit. It relates total value product to the amount of input and total cost to the amount of output (Doll and Orazem, 1984).

The simple profit model can be expressed algebraically as:

$P_Y Y - P_X X - TFC = \text{Profit}$ ………………………………………………..Equation 2

Since $Y = f(X)$ then

$P_Y f(X) - P_X X - TFC = \text{Profit}$ …………………………………………..Equation 3

Where P_Y is output price (price of canola seed), Y is output produced (canola seed), P_X is price of input (price of biosolids), X is variable input (biosolids) and TFC stands for total fixed cost.

To maximize the above profit function with respect to the variable input the first derivative would be set to zero as follows:

$\frac{d\,Profit}{dX} = (P_Y)\frac{dY}{dX} - (P_X) = 0$ ………………………………………………………….Equation 4

Since $\frac{dY}{dX} = MPP$ stands for marginal physical product of input X (biosolids), and rearranging

P_Y MPP = P_X and hence P_Y MPP = VMP, which is the value of marginal physical product equals to input price (price of biosolids).

$(VMP = P_X) = (P_Y \frac{dY}{dX})$ ………………………………………………..Equation 5

In this case the environmental input (biosolids used as fertilizer substitute) enters the production process to produce a single marketed output (canola seed).

Equation 5 would be used to value biosolids as environmental goods in producing canola seed in the production process.

8.1.2. Valuation of biosolids

Valuing dewatered biosolids using the productivity change method

In valuing dewatered and composted biosolids using the productivity change method, it was assumed that the dewatered biosolids were obtained freely and the production costs associated with the growing of canola was not taken into account.

A production function curve for the response of canola seed yields at various dewatered biosolids application rates was estimated and fitted to quadratic function (Fig. 8.1). The marginal product of dewatered biosolids was calculated by taking the first derivatives of the quadratic equation as follows:

The relationship between canola seed yield and dewatered biosolids application rates was estimated and fitted to the quadratic equation ………$y = - 0.0012x^2 + 0.129x + 1.032$ ………..Equation 6

Taking the first derivative of the production function (Equation 6) with respect to dewatered biosolids applications (input) gives $\frac{dy}{dx} = -0.0024x + 0.129 = MPP$………. Equation 7

Equation 7 represents the change in canola seed yield resulting from a unit increment in dewatered biosolids application rates. It is the slope of the production function which measures the amount that total canola seed yield increases or decreases as dewatered biosolids application rates increases. The maximum biosolids application rate that can yield optimum canola seed can be calculated by solving for x in Equation 7.

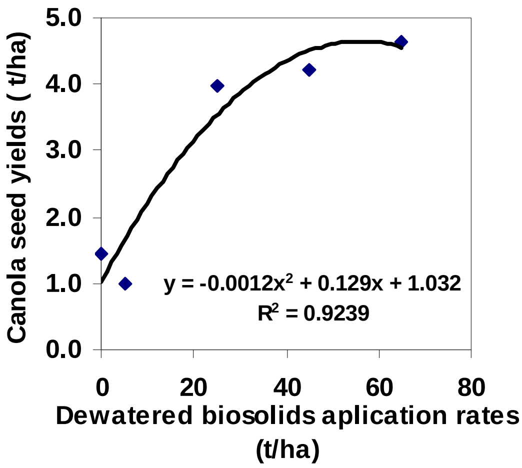
$\frac{dy}{dx} = - 0.0024x + 0.129 = 0$ and the value of x equals to 54 t/ha dewatered biosolids. Hence, to obtain the maximum canola seed yield at the maximum dewatered biosolids rate (54 t/ha), this value (the maximum dewatered biosolids rate) was substituted in Equation 6 and solving for y gives 3.7 t/ha canola seed yield without taking into account the intercept (1.032) from equation 6. Since the intercept refers to the value of canola seed yield obtained at zero biosolids application rate (the canola seed yield from the control plot), it was subtracted from equation 6 to distinguish the effect of biosolids only.

Thus, using the farm gate price of canola seed around Melton which was Aus $ 470/tonne of canola seed, the price of a one tonne of dewatered biosolids was calculated as follows:

Price per tonne of biosolids = (maximum canola seed yield/ha × Price/t of canola seed)/ maximum dewatered biosolids rate [(3.7 × Aus $470)/ 54] and this gave Aus $32.

Therefore, the estimated value per tonne of dewatered biosolids would be Aus $ 32

Similar to the procedures employed in this study, Maler (1991) distinguishes between applications of the production function approach. When production of y is measurable and either there is a market price for this output (P_y) or one can be imputed, then determining the marginal value of the environmental good could be possible.



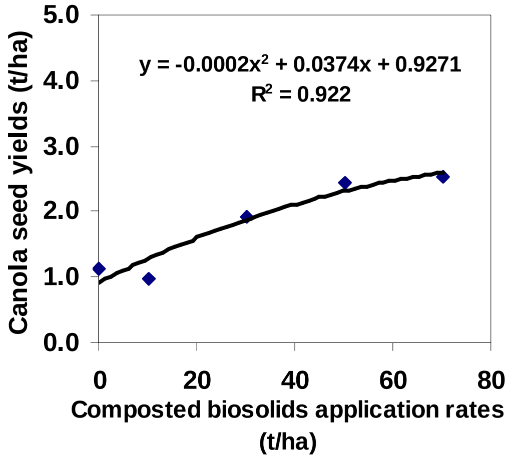


Figure 8.1 Estimated production function curves for the response of canola seed yield to various dewatered biosolids and composted biosolids application rates (t ds/ha) in a field experiment. Each data points indicate means of triplicate measurements for canola seed yield in 2006.

Valuing composted biosolids using the productivity change method

Similar procedures were followed for the valuation of composted biosolids in the production of canola seeds in the field experiment. The response of canola seed yield to composted biosolids applications was estimated and fitted to the production function equation, $y = -0.0002x^2 + 0.0374x + 0.9271$……… Equation 8

Taking the first derivative of Equation 8 with respect to composted biosolids application rates (input) gives $\frac{dy}{dx} = -0.0004x + 0.0374 = MPP$…………………. Equation 9

The maximum composted biosolids application rates that can give optimum canola seed yield was calculated by solving for x from Equation 8 below:

$-0.0004x + 0.0374 = 0$ and gives 94 t/ha composted biosolids. Hence, to obtain the maximum canola seed yield at the maximum composted biosolids rates (94 t/ha), this value (the maximum composted biosolids rate) was substituted in Equation 8 and gives 1.8 t/ha canola seed yield without taking into account the intercept 0.927 from equation 8. The farm gate price of canola seed was Aus $ 470 /tonne of canola seed, the price of a one tonne of composted biosolids was calculated as follows:

Price per tonne of biosolids = (maximum canola seed yield t/ha × Price/t of canola seed)/ maximum composted biosolids rate; (1.8 t/ha × Aus $470)/ 94 t/ha and this gave 9 Aus $ therefore, one tonne of composted biosolids was valued as Aus $ 9.

8.2. Scenario 2 Nutrients from biosolids as fertilizer replacement value

A grower is likely to be willing to pay for biosolids if the product increases overall net returns. An increase in net returns can be achieved by reducing production costs and/or increasing crop yield or quality. Growers and farmers usually have to purchase fertilizers and liming materials, however these can partially be replaced by nutrients from biosolids. The extent to which biosolids provide plant nutrients and/or liming capacity provides an alternative basis for valuing biosolids.

The economic value of biosolids depends on the concentration of the available nutrients (N, P, and K) and the quantity of potentially mineralizable nitrogen and phosphorus contained in the biosolids within a given period of time. Hence, analysis of the plant nutrients contained in the various biosolids forms the basis for assessing the economic value of biosolids.

In the case of scenario 2, dewatered and composted biosolids samples were analysed for total and available plant nutrients and the fertilizer values of biosolids were determined based on its nitrogen, phosphorus and potassium content. Table 8.1 and 8.2 show the average plant nutrients (N, P and K) contained per tonne of dewatered and composted biosolids which were reported on dry weight basis.

The economic values of the biosolids were estimated from the market price of Urea (46% N), triple super phosphate (41% P_2O_5) and sulphate of potash (41% potassium) respectively.

The prices of each of the three plant nutrients Aus$1.70/kgN, 3.37/kg P and 1.20/kgK respectively were calculated from 40 kg bags of urea and triple super phosphate and 1 kg bag of sulphate of potash fertilizers market prices which were sourced from Bacchus Marsh farm supplies in Victoria. Though farmers often buy fertilizers in bulk to get discount, the above prices were used to estimate the value of one tonne of dewatered biosolids and composted biosolids.

Hence, the plant available NPK values contained in a one tonne of biosolids was calculated by multiplying with the unit price for each of the nutrients NPK and added together (Table 8.1 and 8.2). Therefore, as indicated in table 9.1 the value of one tonne of biosolids was estimated to be Aus $20.54 and Aus $13.25 for dewatered and composted biosolids respectively.

Table 8.1 Average quantity of plant available nutrients (NPK) contained in dewatered biosolids (kg/t) and the price/kg and the nutrient values of dewatered biosolids price/tonne of biosolids (in Aus$).

Plant available Nutrients	Market price/kg of nutrients (Aus$)	Quantity of available nutrient/tonne of biosolids (kg/t)	Value of each nutrients in Aus $ (price/t of dewatered biosolids)
N	1.70	10.2	17.34
P	3.37	0.69	2.32
K	1.20	0.73	0.88
Price/tonne of dewatered biosolids	-	-	20.54

As per table 4.1 available phosphorus contained in the biosolids was extracted using the Olsen-extraction technique and analysed by flow injection analysis, whereas, exchangeable potassium was extracted using 1M NH4Cl and determined using ICP-MS. Total nitrogen in biosolids was determined using the Leco FP auto carbon and nitrogen analyzer and the inorganic-N fractions were determined using the cadmium reduction technique in a flow injection analyzer. The inorganic-N fractions were subtracted from the total nitrogen concentration to obtain the organic-N component. Hence, 15% and 10% organic-N mineralization rates for anaerobically digested dewatered and composted biosolids were assumed and the inorganic-N values were determined and added to the previously calculated inorganic-N component and the total inorganic-N was calculated for both biosolids types.
Nutrient analyses were reported on dry weight basis for each of the biosolids types.

Table 8.2 Average quantity of plant available nutrients (NPK) contained in composted biosolids (kg/t) and the price/kg and the nutrient values of composted biosolids price/tonne of biosolids (in Aus$).

Plant available Nutrients	Market price/kg of nutrients (Aus$)	Quantity of nutrient/tonne of biosolids (kg/t)	Value of each nutrients in Aus $(price/t of composted biosolids)
N	1.70	3.08	5.24
P	3.37	0.76	2.56
K	1.20	4.54	5.45
Total Price of NPK/tonne of composted biosolids	-	-	13.25

A similar, Roka *et al.* (2004) used the unit prices of commercially available products and multiplied it by the quantities of plant nutrients and lime equivalents in the biosolids to compute the per-tonne value of biosolids. The economic value of biosolids nutrients N, P, K and dolomite lime in one tonne of the particular biosolids was estimated to be Aus $16.80 which not only depends on the nutrient composition of the material, but also on the application rate. They suggested that economic valuation of biosolids as a resource needs to be broadened to include micronutrients, since most biosolids contain micronutrients in a natural "chelated" form whereas commercially available chelated products can be expensive. Hence, this would enhance the demand for future agricultural land application of biosolids.

Consistent to the findings of this study, several case studies also showed that the first year economic value of nutrients derived from biosolids in the Coastal Plains and Piedmont regions of Virginia range from Aus $50 to Aus $100 per acre on pasture land and Aus $100 to Aus $140 per acre on corn, small grains, and soybean land (one acre is equivalent to 0.4 hectare) (Faulkner, 2001).

8.3. Comparisons between the productivity and nutrient replacement techniques

The results indicated that the value of a one tonne of dewatered biosolids estimated using the productivity change method was significantly greater than the value estimated using the nutrient replacement approach. However, in estimating the value of a one tonne of composted biosolids, both the productivity and the nutrient replacement techniques relatively gave similar results (Table 8.3). Dewatered biosolids had significantly higher value than composted biosolids in both cases of the scenarios, this was due to the higher concentration of inorganic-N fractions contained in dewatered biosolids which significantly contributed to the increase in the price of nitrogen in both scenarios (Table 8.1 and 8.2). The higher canola seed yield increments observed in dewatered biosolids treated plots (Fig. 9.1) was the evidence for the higher response of canola to high inorganic-N supply from dewatered biosolids than composted biosolids treated canola plots.

Table 8.3 Average quantity of plant available nutrients (NPK) contained in composted biosolids (kg/t) and the price/kg and the nutrient values of composted biosolids price/tonne of biosolids (in Aus$).

	Estimated values of biosolids (in Aus $)	
Biosolids types	Productivity approach	Nutrient replacement approach
Dewatered biosolids	32	20.54
Composted biosolids	9	13.25

The quantity of canola seed oil produced using the application of the maximum (54 and 94 t/ha) dewatered biosolids and composted biosolids per hectare were estimated using the data listed in chapter 4 (Table 4.4). Table 4.4 shows the concentrations of canola seed oil obtained at various dewatered biosolids and composted biosolids application rates. Hence, two equations which describe the relationship between dewatered biosolids and composted biosolids applications and canola seed oil concentrations were estimated and fitted to a linear model, $Y = - 0.1273x + 46.2$ and $Y = - 0.0386x + 46.2$ and these equations were used to determine the quantity of canola seed oil per hectare that can be produced at the maximum dewatered biosolids and composted biosolids applications. Table 8.4 shows the total value of canola seed oil per hectare produced from the maximum dewatered biosolids and composted biosolids applications.

Table 8.4 The energy value of canola oil/ ha produced by applying the optimum biosolids rates/ha/yr using canola as energy crop under field conditions

Biosolids type	Maximum biosolids rates (t/ha)	Canola seed yield (t/ha)	Oil concentration (%)	Total oil yield (t/ha)	Energy value of total oil produced MJ/ ha
Dewatered	54	3.7	39.3	1.5	59,670
Composted	94	1.8	42.6	0.8	31,824

The quantity of canola seed oil produced per hectare of land at the maximum dewatered biosolids application was considerably higher than the quantity of seed oil produced from the maximum composted biosolids applications (Table 8.4).

Further, to estimate the energy value of pure canola oil that can be produced per hectare of land under the maximum dewatered and composted biosolids application rates, the heat of combustion of canola oil was used. The heat of combustion measures the energy content of a fuel which is the key property of a biodiesel that determines the appropriateness of this material as an alternative to diesel fuel.

According to studies conducted by (Lang *et al*. 2001; Gerhard *et al*. 2005) the values of the gross heat of combustion for pure canola oil was 39.78 MJ/kg of oil, whereas the heat content of a diesel fuel was approximately 45 MJ/kg and hence the heat of combustion of pure canola oil (39.78MJ/kg) was used as a multiplier to estimate the total energy value of canola oil that can be produced per hectare of land at the maximum dewatered biosolids and composted biosolids applications rates (Table 8.4). Table 8.4 shows that the energy value of total canola oil produced per hectare of land using the maximum dewatered biosolids was 59,670 MJ/ha which was significantly higher than the corresponding energy value (31,824 MJ/ha) of the canola oil produced using the maximum composted biosolids applications.

8.4. Policy implications

Estimating the economic value of biosolids as environmental goods could be a sensible approach particularly for crop growers who may consider replacing part of the plant nutrients requirements normally obtained from commercial fertilisers with nutrients from biosolids. It enhances the efficient utilization of biosolids and municipal solid wastes and promotes recycling and use of the various nutrients contained in the biosolids particularly in the agribusiness sector. Water authorities will be encouraged to adopt a new biosolids policy and install improved dewatering and treatment facilities to produce cleaner biosolid products with very low pathogen levels which can be sold to either the agribusiness sectors or to crop growers at reasonable prices. This further contributes to sustainable management of biosolids in Victoria.

Results of the study clearly demonstrated that dewatered biosolids had greater value than composted biosolids, evidenced by the significantly higher concentrations of total nitrogen levels in dewatered biosolids which was reflected in the considerably higher canola seed oil yield recorded in dewatered biosolids treated canola plots than composted biosolids treated plots.

It is a well recognized fact that the application of biosolids to crop land increases soil organic matter content, improves soil structure and water infiltration **(**Joshua *et al*., 1998; Johansson *et al*., 1999), and improves soil fertility and productivity (Bhogal *et al*., 2003). The nutrient residue in biosolids amended soil can also have long term benefits and may be utilized by subsequent crops, however, nutrients from biosolids can also be lost through various routs such as organic matter degradation during composting could result in carbon dioxide and ammonia gaseous emissions (Paillat *et al*., 2005). Soil organic matter and microbial decay releases carbon dioxide (Janzen, 2004). Microbial transformation of nitrogen in soils and manures also generates nitrous oxide (Smith & Conen 2004).

Therefore, taking all these into account, Western Water at Surbiton Park (WWSP) can possibly incorporate their biosolids either at the optimum (25 t/ha) dewatered biosolids rates and produce 0.7 t/ha canola seed oil or alternatively they can incorporate the computed maximum dewatered biosolids application rate (54 t/ha) which exceeds the crop N requirement onto their land using canola as energy crop and can produce 1.5 t/ha of canola seed oil which would be equivalent to 59,670 MJ from a hectare of land**.** In this regard the use of canola and other crops requiring high nutrients would be advantageous in order to efficiently exploit the nutrients from land applied biosolids.

Therefore, anaerobically digested dewatered biosolids generated from WWSP could be used for agricultural land application purposes without passing the composting processes since composting incurs additional costs and expenses.

The economic valuation technique employed in this study, did not take into account the production costs to produce the canola seed, conversely it would also be important to take into consideration that biosolids not only supply N, P and K but also biosolids have other side benefits such as the provision of plant micronutrients (Cu, Zn, Mn), enhancement of soil microbial populations, supply of organic matter and improvement of structural stability of the soil which were not included in the nutrient replacement valuation technique. These side benefits may increase the future demand for biosolids as valuable resource for plant nutrients and contribute for the wider acceptance of beneficial use of biosolids by crop growers as an alternative to conventional fertilizers.

Although, the value of biosolids depends on the quantity of plant available nutrients contained in the biosolids, the type of crop species that a grower chooses to supply in the market could also affect the price of biosolids, since different crop types do have different market prices which could inturn affect the marginal physical products of the biosolids. Hence, crop growers need to choose high valued crops so as to obtain maximum profit margin from land applied biosolids.

Biosolids are valuable resource, because of the nutrients and organic matter contained in it. It is from this perspective that this chapter quantified the value of biosolids in monetary equivalence.

9

CHAPTER 9. CONCLUSIONS

This study has shown that dewatered biosolids and composted biosolids applications significantly increased the yield and yield components of canola and oats. The number of branches, pods per plant and plant height for canola and the number of grains per panicle and height of the oat crop were increased following biosolids applications. These increases in the yield components significantly contributed to the seed and biomass yields increments of both crops, evidenced by the significant positive correlations observed between seed and plant biomass, between the number of pods and branches for canola and between seed yield and plant height in the oat crop. The following observations were made:

Canola seed yields were optimum at 25 t/ha (1055 kg total N/ha) dewatered biosolids and at 30 t/ha (432 kg total N/ha) composted biosolids loading rates in the 2006 experiment, while, the optimum oats seed yield in the 2006 trial were observed at the 25 t/ha dewatered biosolids and 50 t/ha (720 kg total N/ha) composted biosolids rates, respectively

In the 2007 trial, the optimum canola seed yield were observed at the 45 t/ha (1899 kg total N/ha) dewatered biosolids and 50 t/ha composted biosolids rates, respectively. The optimum dewatered biosolids and composted biosolids rates in 2007 were shifted for both biosolids types, these increases might be due to inter seasonal variations in the response of the two crops to biosolids nutrients

At the higher biosolids applications canola and oats seed and dry biomass yields were greater than fertilized and unamended control plots for both crops

Canola and oats seed and biomass yields obtained from dewatered biosolids treatments were significantly higher than those recorded from composted biosolids treated plots. The response of canola to biosolids applications in terms of yield and yield components was higher than oats crops for both biosolids types in both years of the experiments

The applications of both biosolids types increased the concentrations of total N, P and S in canola seed, however seed oil concentration was decreased as biosolids application rates increased and was negatively correlated with the concentrations of total N in canola seed negatively impacting the quality the oil, nevertheless, the significant increase in the quantity of seed yield due to N from biosolids compensated for any decrease in seed oil concentration by increasing the quantity of seed oil

Although, levels of Cu, Zn and Fe determined using both ICP-MS and XRF in biosolids amended soil showed an increasing trend as both types of biosolids applications increased, XRF determined total metals levels as expected were significantly higher than their corresponding ICP-MS determined values

Slight increments in the values of the ratio of ICP-MS to XRF values for most of the heavy metals at higher biosolids application rates (45 and 65 t/ha dewatered and 50 and 70 t/ha composted biosolids rates) were observed, indicating that HNO_3/H_2O_2 extraction procedure prior ICP-MS analysis was to some extent enhanced by the organic matter content of the amended soil

Though ICP-MS had higher sensitivity and lower detection limits than XRF, XRF is a better option for total metal analysis particularly for soil analysis due to its shorter preparation time and ease of instrument operation. In addition to this, the smaller the standard deviations and consistency of the results and the higher the recoveries of the standard reference materials, XRF would be the preferred option for routine laboratory analysis of soil samples

The total and DTPA extractable concentrations of Cu, Zn, Mn, Fe, Co and Pb in the amended soil increased following dewatered biosolids and composted biosolids applications. However, total Cu and Zn concentrations determined by XRF even at the highest 65 and 70 t/ha dewatered biosolids and composted biosolids amended plots did not exceed the maximum EPA permitted ceiling limits for soils receiving biosolids used for crop production

A significant change in soil pH following biosolids application was observed, where dewatered biosolids lowered the soil pH, while composted biosolids increased the pH of the soil at higher biosolids application rates

The study has also clearly demonstrated that the concentrations of DTPA extractable soil Zn and Fe residue recorded after two years for canola-oats rotation were significantly higher than the levels found in the oats-canola cropping sequence

This could be due to canola's efficiency in terms of absorbing the soluble metals from the soil solutions than the oat crop. It is also speculated that due to the organic acids released by canola roots which could possibly decompose the organic matter thereby increasing the soluble metals into the soil system. Moreover, when the canola roots residues decompose they may add organic acid into the soil which may convert the insoluble metal fractions into soluble forms and hence raising the available metal fractions in the canola-oats cropping sequence, or this may probably be due to the lower yield of oats recorded in year two due to rust infestation of the crop

Significant positive correlations between DTPA extractable metals and their respective concentrations in canola and oats tissue were also observed, indicating the use of DTPA extractable heavy metals as reliable estimate of plant available heavy metals in soil

Among the metals investigated in this study, the uptake of Cu and Zn by canola crop was significantly higher than the control plots and was linearly related to application rate. On the other hand Cu and Zn uptake by oats showed a plateau type response

In addition to this, the concentration of Cu and Zn uptake by canola was significantly higher than the levels observed in oats leaves, hence the use of canola in rotation with other cereal crops in biosolids amended soil could be used to extract some of the soluble Cu and Zn from the soil

The solubility of heavy metals and their subsequent uptake by plants mainly depends on soil properties such as pH, organic matter, CEC and clay content of the receiving soil. Thus, periodic monitoring of these soil properties in biosolids amended soil would be important with emphasis given to soil pH. For instance if biosolids are repeatedly applied in sandy acidic soils, the total Cu and Zn levels in biosolids amended soils need to be monitored on a regular basis to protect the soil quality

If biosolids are added at agronomic rate (1NLBAR for canola which is equivalent to 10 t/ha ds), dewatered biosolids can consecutively be applied for 64 years whereas for the oat crop since 1NLBAR was equivalent to 11 t/ha ds, thus dewatered biosolids can be applied for 45 successive years to reach the Victorian EPA maximum ceiling limits for Zn. For reasons of P management, biosolids should be applied once in a crop rotation therefore in reality it may take greater than 200 years in total to reach the soil limits. In addition, the metal residue models are based on a cultivation depth of 10 cm, in practice the plough depth may be 25 cm, so the maximum concentration might not be attained for 500 years in practice

Even though, nutrients and metals residues accumulated in soil and plant also partly depends on previous history, crop disease and other factors, the oats-canola cropping sequence could be suggested as a better cropping option to relatively utilize nutrients from the biosolids than the canola-oats cropping sequence since it significantly reduced the Zn and Fe concentrations accumulated in the amended soil, however, further study on the impact of crop rotation on nutrients levels accumulated in biosolids amended soil need to be conducted

As expected, the concentrations of total and extractable forms of N and P in soil and in plants also increased significantly following dewatered biosolids and composted biosolids loading rates. Dewatered biosolids and composted biosolids applications also increased the S status of the soil. However, a considerable amount of total and Olsen-extractable P remained in the biosolids amended soil after crop harvest and this exceeded the agronomic requirements of both crops. Nonetheless, since the biosolids receiving soil had a clay content of 31 %, with pHw of 6.5, CEC (7 meq/kg) and organic carbon (2.6 %), it may not pose any threat to soil or ground water contamination since the clay minerals are likely to bind with P before leaching could occur

The accumulated P could be used for producing a subsequent crop through crop rotation. The available soil P also supports weed growth which would reduce runoff availability but compromise crop quality

The observed NO_3-N concentration leaching down in the 20 cm soil depth of the canola plots was significantly lower than the levels found in the oats plots, confirming canola's greater capacity to take up NO_3-N from biosolids amended soil. In this study, the risk of ground water contamination due to leaching of NO_3-N down the soil profile due to biosolids application was negligible and did not cause any problem, since most of the NO_3-N was accumulated on the top 20 cm soil depth, however, as single measurement of NO_3-N at the end of the growing period was taken, it is suggested that NO_3-N measurements need to be taken at different season of the year for a better understanding and conclusion of NO_3-N leaching potential in biosolids amended soil. Moreover, in the inorganic fertilizer treated plots, the fertilizers were not incorporated into the soil, and it is expected that some portion of the nitrogen may have been lost through NH_3-N volatilization and denitrfication processes and was not compared in the assessment of NO_3-N leaching

From the results of the study, it can be suggested that the two biosolids types had different physical and chemical properties and behaved differently, and thus the relevance and assumption of using NLBAR (nitrogen limited biosolids application rate) may not be valid for all types of biosolids products, and underestimated the organic nitrogen mineralization rates, furthermore nutrient release properties of the two biosolids could be different and depends soil temperature, moisture, crop and soil types. Hence, it is suggested that biosolids application should take into account the soil and biosolids type and the specific site characteristics of the area receiving the biosolids

The correlations between total N in soil and total N and NO_3-N in canola and oats were significant. Likewise, significant positive correlations were also noted between total P in soil and extractable P in canola and oats. In addition to this, soil test values for total N, NO_3-N and Olsen-P could be used to predict the plant available and uptake of these nutrients in biosolids amended clay loam soil

Similar to the findings of the heavy metals, significantly higher concentrations of Olsen-P and total-N residue accumulated in the canola-oats cropping sequence than in the oats-canola crop rotation in both dewatered and composted biosolids treated plots.

Total N and acetic acid extractable P in canola leaves were significantly higher than in oats leaves indicating that canola extracted more N and P from biosolids amended soil than oats, indeed due to its high nutrient (N, P and S) requirements, canola could thus be used as a clean up crop particularly in biosolids amended land

The oats-canola cropping sequence could serve as an alternative option to efficiently utilize the residual nutrients in a biosolids amended soil and reduce the build up of nutrients and heavy metals in biosolids receiving soil. Hence, growers may benefit from the N and P residues from biosolids by adopting an oats-canola rotational cropping system to effectively utilize the N from biosolids and employ the accumulated P in the receiving soil. However, it can also be suggested that long term crop rotation trials using various crops need to be conducted in order to further verify the impact of cropping sequence on nutrients and heavy metals accumulated in biosolids amended soil. Moreover, to efficiently remediate the accumulated P in the biosolids amended land, it can also be suggested that studies particularly focusing on the phytoextraction capacity of both Australian indigenous and exotic plant species need to be further investigated

Depending on the P status of a particular soil, the future use of biosolids limited phosphorus application rates (PLBAR) under Victorian agricultural context needs to be further investigated for sustainable management of P on biosolids amended land.

Undoubtedly, the mineralization rates of organic-N and P contained in biosolids depends on various environmental factors, of which soil moisture, temperature, biosolids characteristics and microbial activity are the key elements and hence in order to safeguard excessive soil residue and the leaching of N and P down the soil profile, knowledge of the potentially mineralizable components of the organic-N and P in biosolids would be an important subject that entails further investigation

It would be important to adopt a systems approach to the application of the two crops selected for trial to determine if there are economic benefits in having higher biosolids loadings on smaller areas of land supporting canola compared with lower loadings supporting oats. Opportunities for breeding crops specifically for biosolids utilization and biofuel processing should also be explored as this experiment has demonstrated that both crop selection and type of biosolids impacted nutrient uptake, accumulations and dry matter production

After year one and year two successive land applications of dewatered biosolids and composted biosolids, the level of pathogens surviving in the amended soils were very low compared with the EPA Vic. guidelines for grade T1 “unrestricted” use of biosolids which requires an E.coli measure of < 100 per gram of dry biosolids and < 1 Salmonella per 5 g of sample. Hence, the biosolids complied with the bacterial requirements so far tested for unrestricted agricultural use

Estimating the economic value of biosolids as environmental goods could be a sensible approach particularly for crop growers who may consider replacing part of the plant nutrients requirements with nutrients from biosolids than with conventional fertilizers. It enhances the efficient utilization of biosolids by the agribusiness sectors. Waste Water treatment authorities will also be encouraged in recycling and use of the various nutrients contained in the biosolids and further formulate and adopt a new biosolids policy and install improved dewatering and treatment facilities to produce cleaner biosolids products which can be sold to either the agribusiness sectors or to crop growers at reasonable prices. This further contributes to sustainable management of biosolids in Victoria

Results of the study clearly demonstrated that dewatered biosolids had greater value than composted biosolids, this was evidenced by the significantly higher concentrations of total nitrogen levels in dewatered biosolids than in composted biosolids which was reflected in the considerably higher canola seed yield recorded in dewatered biosolids treated canola plots than composted biosolids treated plots in the field experiment. Dewatered biosolids gave significantly higher quantity of canola seed oil and had greater value than the amount of seed oil produced from composted biosolids

Since composting incurs additional costs and is expensive, anaerobically digested dewatered biosolids generated from WWSP, without passing the composting process may possibly be incorporated at the optimum (25 t/ha or 1055 total N/ha) rates to produce 0.7 t/ha canola seed oil or alternatively, the computed maximum dewatered biosolids application rate (54 t/ha or 2279 total N/ha)) which exceeds the crop N requirement can be incorporated to produce 1.5 t/ha of canola seed oil which would be equivalent to 59,670 MJ from a hectare of land. In this regard the use of canola and other crops requiring high nutrients would be advantageous in order to efficiently exploit the nutrients from land applied biosolids

The environmental valuation techniques employed in this study, did not take into account the production costs incurred to produce the canola seed, however it would also be important to take into account that biosolids not only supply N, P and K but also biosolids could provide other side benefits such as the provision of plant micronutrients (Cu, Zn, Mn), enhancement of soil microbial populations, supply of organic matter and improvement of structural stability of the soil which were not included in the valuation process. Knowledge of the various side benefits of biosolids and its alternative use as conventional fertilizers would increase the demand for beneficial use of biosolids by crop and livestock farmers which inturn contributes to sustainable management of land applied biosolids

The quantity of plant available nutrients contained in the biosolids and the types of crops that a grower produces would ultimately determine the price (value) of biosolids, and thus to maximize profit and farm income from the application of biosolids, growers need to be selective in choosing high valued crops

To effectively promote the agricultural land application of biosolids in Victoria and improve the process of adoption by the farming community, the collaborative works between researchers and government departments together with the media is crucially important

REFERENCES

ABARE (2003) Australian Crop Report, Number 125. 18 February, 2003. (Australian Bureau of Agricultural and Resource Economics, Canberra).

ABDUL, P. & LLOYD, D. (1985) Pathogen survival during anaerobic digestion: fatty acids inhibit anaerobic growth of Escherichia coli. Biotechnology Letters, 7, 125-8.

ADAM, S. M. & ADAM, W. A. (1979) Effect of soil pH on the growth and mineral content of oats. Pakistan Journal of Scientific and Industrial Research, 22**,** 147-151.

ADEGBIDI, H. G. & BRIGGS, R. D. (2003) Nitrogen mineralization of sewage sludge and composted poultry manure applied to willow in a greenhouse experiment. Biomass Bioenerg., 25, 665-673.

ADRIANO, D. C. (1986) Trace Elements in the Terrestrial Environment, published by Springer-verlag in New York.

ANDRIAN, J. (2003) Phosphorus Nutrition of Canola. Potash and Phosphate Institute of Canada (PPIC)

AKIN, E. W., JAKUBOWSKI, W., LUCAS, J. B. & PAHREN, H. R. (1977) Health hazards associated with wastewater effluents and sludge: microbiological considerations. Health Effects Res. Lab., EPA, Cincinnati, OH, USA.

ALLEN, E. J. & MORGAN, D. G. (1972) A quantitative analysis of the effect of nitrogen on the growth development and yield of oilseed rape. J.Agric. Sci. Camb., 78, 315-324.

ALLOWAY, B. J. (1995) Heavy Metals in Soils Second Edition, published by Blackie Academic & Professional, London p352

ALLOWAY, B. J. & JACKSON, A. P. (1991) The behaviour of heavy metals in sewage sludge-amended soils. The Science of the total environment, 100 151-76.

ALVA, A. K., HUANG, B. & PARAMASIVAM, S. (2000) Soil pH affects copper fractionation and phytotoxicity. Soil Science Society of America Journal, 64, 955-962.

ALVES, M. C., GONZALEZ, A. P., COLODRO, G., PERECIN, J. H. & VAZQUEZ, E. V. (2006) Influence of biosolids rate on chemical properties of an oxisol in sao paulo, Brazil. Communications in Soil Science and Plant Analysis, 37, 2481-2493.

ANDERSEN, M. N., HEIDMANN, T. & PLAUBORG, F. (1996) The effects of drought and nitrogen on light interception, growth and yield of winter oilseed rape. Agriculturae Scandinavica, Sect. B, Soil and Plant Science, 46, 55–67.

ANDERSON, G. & ARLIDGE, E. Z. (1962) The adsorption of inositol phosphates and glycerophosphate by soil clays, clay minerals, and hydrated sesquioxides in acid media. Journal of Soil Science, 13, 216-24.

ANDERSON, M. A. (1997) Long-term copper availability and adsorption in a sludge amended clay loam soil, PhD. dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA.

ANDERSON, M. S. (1959) Fertilizing characteristics of sewage sludge. Sewage and Industrial Wastes, 31, 678-82.

ANDERSON, P. R. & CHRISTENSEN, T. H. (1988) Distribution coefficients of cadmium, cobalt, nickel, and zinc in soils. Journal of Soil Science, 39, 15-22.

ANDERSON, W. K. & MCLEAN, R. (1989) Increased responsiveness of short oat cultivars to early sowing, nitrogen fertilizer and seed rate. Aust. J. Agric. Res., 40, 729–744.

ANGLE, J. S., CURRIE, V. C. & HILL, R. L. (2003) Biosolids application to soybeans and effects on input and output of nitrogen. Agriculture, Ecosystems and Environment 97, 345–351.

ANGUS, J. F., DESMARCHELIER, J. M., GARDNER, P. A., GREEN, A., HOCKING, P. J., HOWE, G. N., KIRKEGAARD, J. A., MARCROFT, S., MEAD, A. J., POTTER, T. D., RYAN, M. H., SARWAR, M., VAN HERWAARDEN, A. F. & WONG, P. T. W. (1999) Canola and Indian mustard as break crops for wheat. Proceedings of the 10th International Rapeseed Congress, Canberra, September 26-29, 1999. (Eds. N. Wratten & P. Salibury).

ANGUS, J. F., KIRKEGAARD, J. A. & PEOPLES, M. B. (2001) Rotation, sequence and phase: research on crop and pasture systems. Proceedings of 10th Australian Agronomy Conference, Hobart,February 2001.

ANGUS, J. F., VAN HERWAARDEN, A. & FISCHER, R. A. (1989) Predicting the yield response of wheat to topdressed nitrogen. Proceedings 5th Australian Agronomy Conference, Perth, p.550.
.
ANTOLIN, M. C., PASCAUL, I., GARCIA, C., POLO, A. & SANCHEZ-DIAZ, M. (2005) Growth, yield and solute content of barley in soils treated with sewage sludge under semiarid Mediterranean conditions. Field Crops Res., 94, 224-237.

ANTONIADIS, V. & ALLOWAY, B. J. (2002) The role of dissolved organic carbon in the mobility of Cd, Ni and Zn in sewage sludge-amended soils. Environmental Pollution (Oxford, United Kingdom), 117, 515-521.

ANTONIADIS, V. & ALLOWAY, B. J. (2003) Evidence of heavy metal movement down the profile of a heavily-sludged soil. Communications in Soil Science and Plant Analysis, 34, 1225-1231.

APHA, (1995) Standard methods for the examination of water and waste water, 19th edn. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, DC.

APLING, E. C. (1996) The Oat Crop: Production and Utilization, edited by Robert W. Welch.

ARBESTAIN, C. M., EGIARTE , G., ALONSO , A., RUı´Z-ROMERA , E. & PINTO, M. (2005) Effect of repeated applications of sewage sludge on the fate of N in soils under Monterey pine stands. Forest Ecology and Management 216 257–269.

ARMSTRONG, K. W., RUITER, J. D. & BEZAR, H. (2004) Chapter X - Fodder Oats: Fodder Oats in New Zealand and Australia - History, Production and Potential. Fodder Oats: a world overview. Edited by J M Suttie and S G Reynolds. Plant Production and Protection Series No. 33. Food and Agricultural Organisation of the United Nations, Rome. ISBN 92-5-105243-3. http://www.fao.org/docrep/008/y5765e/y5765e0e.htm

ARNESEN, A. K. M. & SINGH, B. R. (1999) Plant uptake and DTPA-extractability of Cd, Cu, Ni and Zn in a Norwegian alum shale soil as affected by previous addition of dairy and pig manures and peat. *Can. J. Soil Sci. ,* 78**,** 531-539.

ARTIOLA, J. F. & PEPPER, I. L. (1992) Denitrification activity in the root zone of a sludge-amended desert soil. Biology and Fertility of Soils, 13, 200-205.

AUSTRALIAN PLANT SOCIETY MAROONDAH (2001) Flora of Melbourne A Guide to the Indigenous Plants of the Greater Melbourne Area.Hyland House, Melbourne

AUSTRALIAN STANDARD 4454 (2003) Composts, soil conditioners and mulches, prepared by Committee CS-037, Garden Soils and Potting Mixes.

AVERY, S. M., MOORE, A. & HUTCHISON, M. L. (2004) Fate of Escherichia coli originating from livestock faeces deposited directly onto pasture. Letters in applied microbiology, 38, 355-359.

BADARUDDIN, M. & MEYER, D. W. (1990) Green-manure legume effects on soil nitrogen, grain yield, and nitrogen nutrition of wheat. Crop Science, 30, 819-825.

BAKER, A. J. M. (1981) Accumulators and excluders - strategies in the response of plants to heavy metals. Journal of Plant Nutrition, 3, 643-54.

BAILEY, L. D. (1990) The effects of 2-chloro-6 (trichloromethyl)-pyridine (N-serve) and N fertilizers on productivity and quality of Canadian rape cultivars. *Can. J. Plant. Sci.,*70**,** 979 - 986.

BAKHSH, A., KANWAR, R. S. & KARLEN, D. L. (2005) Effects of liquid swine manure applications on NO3–N leaching losses to subsurface drainage water from loamy soils in Iowa Agriculture Ecosystems and Environment, 109, 118–128.

BALDWIN, A., BROWN, T.A., BECKETT, P.H.T., ELLIOTT, G.E.P., (1983).The forms of combination of Cu and Zn in digested sewage sludge.W ater Res. 17, 1935–1944.8)

BALDWIN, K. R. & SHELTON, J. E. (1999) Availability of heavy metals in compost-amended soil. Bioresource Technology, 69, 1-14.

BANUELOS, G. S., PASAKDEE, S., BENES, S. E. & LEDBETTER, C. A. (2007) Long-Term Application of Biosolids on Apricot Production. Communications in Soil Science and Plant Analysis, 38, 1533-1549.

BARROW, N. J. (1987) The effects of phosphate on zinc sorption by a soil. Journal of Soil Science, 38, 453-459.

BARRY, G. A., CHUDEK, P. J., BEST, E. K. & MOODY, P. W. (1995) Estimating sludge application rates to land based on heavy metal and phosphorus sorption characteristics of soil. Water Research, 29, 2031-2034.

BARRY, G., BELL, M. & PU, G. (2006) Sustainable Biosolids Recycling in South East

Queensland Final Report to Brisbane Water and SEQROC Biosolids Management Project Group. Queensland Department of Natural Resources and Mines and Primary Industries and Fisheries, Australia.

BASTA, N. T. & MCGOWEN, S. L. (2004) Evaluation of chemical immobilization treatments for reducing heavy metal transport in a smelter-contaminated soil. Environmental pollution (Barking, Essex 1987), 127, 73-82.

BASTOS, R. K. X. & MARA, D. D. (1995) The bacterial quality of salad crops drip and furrow irrigated with waste stabilization pond effluent: An evaluation of the WHO guidelines. Water Science and Technology, 31, 425-430.

BAVEYE, P., MCBRIDE, M. B., BOULDIN, D., HINESLY, T. D., DAHDOH, M. S. A. & ABDEL-SABOUR, M. F. (1999) Mass balance and distribution of sludge-borne trace elements in a silt loam soil following long-term applications of sewage sludge. Science of the Total Environment, 227, 13-28.

BECKETT, P. H. T. (1989) The Use of Extractants in Studies on Trace Metals in Soils, Sewage Sludges and Sludge-Treated Soils. Adv. Soil Sci., 9, 143–176.

BEEGLE, D., FOX, R., ROTH, G. & PIEKIELEK, W. (1994) Pre-side dress soil nitrate test for corn. Agronomy Facts 17. Pennsylvania State Univ.

BELL, M. (2007) Key findings and recommendations on Nutrient issues from National Biosolids Research Program. Biosolids specialty III conference, Melbourne

BELL, P. F., JAMES, B. R. & CHANEY, R. L. (1991) Heavy metal extractability in long-term sewage sludge and metal salt-amended soils. Journal of Environmental Quality, 20, 481-486.

BENITEZ, E., ROMERO, E., GOMEZ, M., GALLARDO-LARA, F. & NOGALES, R. (2001) Biosolids and biosolids-ash as sources of heavy metals in a plant-soil system. Water, Air, and Soil Pollution, 132, 75-87.

BERGKVIST, P., JARVIS, N., BERGGREN, D. & CARLGREN, K. (2003) Long-term effects of sewage sludge applications on soil properties, cadmium availability and distribution in arable soil. Agriculture, Ecosystems & Environment, 97, 167-179.

BERTHELSEN, B. O., STEINNES, E., SOLBERG, W. & JINGSEN, L. (1995) Heavy metal concentrations in plants in relation to atmospheric heavy metal deposition. Journal of Environmental Quality, 24, 1018-26.

BERTI, W. R. & JACOBS, L. W. (1996) Chemistry and phytotoxicity of soil trace elements from repeated sewage sludge applications. Journal of Environmental Quality, 25, 1025-1032.

BHATTACHARYYA, P., CHAKRABORTY, A., CHAKRABARTI, K., TRIPATHY, S. & POWELL, M. A. (2006) Copper and zinc uptake by rice and accumulation in soil amended with municipal solid waste compost. Environmental Geology (Heidelberg, Germany), 49, 1064-1070.

BHOGAL, A., NICHOLSON, F. A., CHAMBERS, B. J. & SHEPHERD, M. A. (2002) Effects of past sewage sludge additions on heavy metal availability in light textured soils:

implications for crop yields and metal uptakes. Environmental Pollution (Oxford, United Kingdom), 121, 413-423.

BIDWELL, A. M. & DOWDY, R. H. (1987) Cadmium and zinc availability to corn following termination of sewage sludge applications. Journal of Environmental Quality, 16, 438-42.

BILSBORROW, P. E., EVANS, E. J. & ZHAO, F. J. (1993) The influence of spring nitrogen on yield, yield components and glucosinolate content of autumn-sown oilseed rape (Brassica napus). Journal of Agricultural Science, 120, 219-24.

BINDER, D. L., DOBERMANN, A., SANDER, D. H. & CASSMAN, K. G. (2002) Biosolids as nitrogen source for irrigated maize and rainfed sorghum. Soil Science Society of America Journal, 66, 531-543.

BINGHAM, F. T., PAGE, A. L., MAHLER, R. J. & GANJE, T. J. (1975) Growth and cadmium accumulation of plants grown on a soil treated with cadmium-enriched sewage sludge. Journal of Environmental Quality, 4, 207-11.

BITTON, G. (1994) Wastewater Microbiology, Wiley-Liss, Inc., New York.

BJERRE, G. & SCHIERUP, H.-H. 1985. Uptake of six heavy metals by oat as influenced by soil type and additions of cadmium, lead, zinc and copper. Plant and Soil, 88**,** 57-69.

BLACKSHAW, R. E. (1991) Soil temperature and moisture effects on down brome vs. winter canola, wheat and rye emergence. Crop Sci, 31, 1034–1040.

BOLAN, N. S., HEDLEY, M. J. & WHITE, R. E. (1991) Processes of soil acidification during nitrogen cycling with emphasis on legume-based pastures. Plant and Soil, 134, 53-63.

BOSWELL, F. C. (1975) Municipal sewage sludge and selected element applications to soil. Effect on soil and fescue. Journal of Environmental Quality, 4, 267-73.

BOWEN, H. J. M. (1979) Environmental Chemistry of the Elements.

BOWMAN, D. D., LITTLE, M. D. & REIMERS, R. S. (2003) Precision and accuracy of an assay for detecting Ascaris eggs in various biosolid matrices. Water Research, 37, 2063-2072.

BOYCE, T. G., SWERDLOW, D. L. & GRIFFIN, P. M. (1995) Escherichia coli O157:H7 and the hemolytic-uremic syndrome. The New England journal of medicine, 333, 364-368.

BOZKURT, M. A. & YARILGAC, T. (2003) The effects of sewage sludge applications on the yield, growth, nutrition and heavy metal accumulation in apple trees growing in dry conditions. Turkish Journal of Agriculture and Forestry, 27, 285-292.

BRADY, N. C. & WEIL, R. R. (2002) The Nature and Properties of Soil, Prentice Hall.

BRAZAUSKIENE, D.-M., PAULAUSKAS, V. & SABIENE, N. (2008) Speciation of Zn, Cu, and Pb in the soil depending on soil texture and fertilization with sewage sludge compost. Journal of Soils and Sediments, 8, 184-192.

BREMNER, J. M. & KEENEY, D. R. (1966) Determination and isotope-ratio analysis of different forms of nitrogen in soils. III. Exchangeable ammonium, nitrate, and nitrite by extraction-distillation methods. Soil Science Society of America Proceedings, 30, 577-582.

BRENNAN, R. F. & BOLLAND, M. D. A. (2005) Canola takes up more cadmium and phosphorus from soil than spring wheat. Journal of Plant Nutrition, 28, 931-948.

BRENNAN, R. F., MASON, M. G. & WALTON, G. H. (2000) Effect of nitrogen fertilizer on the concentrations of oil and protein in canola (Brassica napus) seed. Journal of Plant Nutrition, 23, 339-348.

BRESLER, E., MCNEAL, B. L. & CARTER, D. L. (1982) Saline and Sodic soils. Springer-Verlag, New York.

BRESLIN, V. T. (1999) Retention of metals in agricultural soils after amending with MSW and MSW-biosolids compost. Water, Air, and Soil Pollution, 109, 163-178.

BREWER, L. J. & SULLIVAN, D. M. (2003) Maturity and stability evaluation of composted yard trimmings. Compost Science & Utilization, 11, 96–112

BRIANSKAIA, A. M. (1966) Antagonistic action of actinomycetes on pathogenic bacteria in the soil. Gigienai sanitariia, 31, 104-105.

BRINKMAN, M. A. & Y.D., R. (1984) Response of three oat cultivars to N fertilizer. Crop Science, 24, 973–976.

BROOS, K., WARNE, M. S. J., HEEMSBERGEN, D. A., STEVENS, D., BARNES, M. B., CORRELL, R. L. & MCLAUGHLIN, M. J. (2007) Soil factors controlling the toxicity of copper and zinc to microbial processes in Australian soils. Environmental Toxicology and Chemistry, 26, 583-590.

BROWN, S., CHANEY, R. & ANGLE, J. S. (1997) Subsurface liming and metal movement in soils amended with lime-stabilized biosolids J Environ Qual 26**,** 724-732

BROWN, S. L., CHANEY, R. L., ANGLE, J. S. & RYAN, J. A. (1998) The phytoavailability of cadmium to lettuce in long-term biosolids-amended soils. Journal of Environmental Quality, 27, 1071-1078.

BROWN, S.L., HENRY, C.L., CHANEY, R., COMPTON, H. & DEVOLDER, P.S. (2003) Using municipal biosolids in combination with other residuals to restore metal-contaminated mining areas. Plant Soil 249:203–15.

BROWN, S., CHANEY, R., HALLFRISCH, J., RYAN, J.A. & BERTI, W.R. (2004) In situ soil treatments to reduce the phyto- and bioavailability of lead, zinc, and cadmium. J Environ Qual 33:522–31.

BRUN, L. A., MAILLET, J., HINSINGER, P. & PEPIN, M. (2001) Evaluation of copper availability to plants in copper-contaminated vineyard soils. Environmental Pollution (Oxford, United Kingdom), 111, 293-302.

BRYAN, F. L. (1977) Disease transmitted by foods contaminated by wastewater. J. Food Protec., 40, 45–52.

BUTKUTE, B., SIDLAUSKAS, G. & BRAZAUSKIENE, I. (2006) Seed yield and quality of winter oilseed rape as affected by nitrogen rates, sowing time, and fungicide application. Communications in Soil Science and Plant Analysis, 37, 2725-2744.

CAMERON, K. C., DI, H. J. & MCLAREN, R. G. (1997) Is soil an appropriate dumping ground for our wastes? Australian Journal of Soil Research, 35, 995-1035.

CAMPBELL, D. J. & BECKETT, P. H. T. (1988) The soil solution in a soil treated with digested sewage sludge. T Soil Sci 39**,** 283-298.

CANOLA COUNCIL OF CANADA (2002) Canola - standards and regulations. http://www.canola-council.org.

CARRINGTON, E. G., HARMAN, S. A. & PIKE, E. B. (1982) Inactivation of Salmonella during anaerobic digestion of sewage sludge. The Journal of applied bacteriology, 53, 331-334.

CAWSE, P. A. (1987) Trace and major elements in the atmosphere at rural locations in Great Britain, 1972–81. In: Pollutant Transport and Fate in Ecosystems, Coughtrey, P. J., Martin, M. H., and Unsworth, M. H., Eds., Blackwell Scientific, Oxford, 89.

CHANDER, K., HARTMANN, G., JOERGENSEN, R. G., KHAN, K. S. & LAMERSDORF, N. (2008) Comparison of methods for measuring heavy metals and total phosphorus in soils contaminated by different sources. Archives of Agronomy and Soil Science, 54, 413-422.

CHANEY, R. L. (1973) Crop and food chain effects of toxic elements in sludges and effluents. 129-141.

CHANEY, R. L. (1983) Potential effects of waste constituents on the food chain, pp. 152-240, J.F. Parr, P.B. Marsh and J.M. Kla (Eds.), Land Treatment of Hazardous Wastes, Noyes Data Corp., Park Ridge, NJ.

CHANEY, R. L. & RYAN, J. A. (1993) Heavy metals and toxic organic pollutants in MSW-composts: research results on phytoavailability, bioavailability, fate, etc. In: Science and Engineering of Composting: Design, Environmental, Microbiological and Utilization Aspects. Eds H A J Hoitink and H M Keener., Renaissance Pub, Worthington, OH.

CHANEY, R. L. (1994) Trace metal movement: Soil-plant systems and bioavailability of biosolids-applied metals, C.E. Clapp, W.E. Larson and R.H. Dowdy (Eds.), Sewage Sludge: Land Utilization and the Environment, American Society of Agronomy, Madison, WI.

CHANEY, R.L., BROWN, S.L., STUCZYNSKI, T.I., et al., (1999). Insitu remediation and phytoextraction of metals from hazardous contaminated soils.Pr esented at US-EPA's Conference: Innovative Clean-Up Approaches: Investments in Technology Development, Results & Outlook for the Future.Nov . 2–4, Bloomingdale, IL.

CHANG, A. C., PAGE, A. L., SUTHERLAND, F. H. & GRGUREVIC, E. (1983) Fractionation of phosphorus in sludge-affected soils. Journal of Environmental Quality, 12, 286-90.

CHANG, A. C., PAGE, A. L. & WARNEKE, J. E. (1987) Long-term sludge applications on cadmium and zinc accumulation in Swiss chard and radish. Journal of Environmental Quality, 16, 217-21.

CHANG, A. C., WARNEKE, J. E., PAGE, A. L. & LUND, L. J. (1984) Accumulation of heavy metals in sewage sludge-treated soils. Journal of Environmental Quality, 13, 87-91.

CHANG, L. L., RAUDENBUSH, D. L. & DENTEL, S. K. (2001) Aerobic and anaerobic biodegradability of a flocculant polymer. Water Science and Technology, 44, 461-468.

CHAPMAN, H. D. (1966) Diagnostic Criteria for Plants and Soils.

CHAUDHRY, F. M. & LONERAGAN, J. F. (1972) Zinc absorption by wheat seedlings and the nature of its inhibition by alkaline earth cations. Journal of Experimental Botany, 23, 552-560.

CHAUDRI, A. M., ALLAIN, C. M. G., BARBOSA-JEFFERSON, V. L. & NICHOLSON, F. A. (2000) A study of the impacts of Zn and Cu on two rhizobial species in soils of a long-term field experiment. . Plant and Soil, 221, 167–179.

CHAUDRI, A. M., ALLAIN, C. M. G., BARBOSA-JEFFERSON, V. L., NICHOLSON, F. A., CHAMBERS, B. J. & MCGRATH, S. P. (2000) A study of the impacts of Zn and Cu on two rhizobial species in soils of a long-term field experiment. Plant and Soil, 221, 167-179.

CHAURET, C., SPRINGTHORPE, S. & SATTAR, S. (1999) Fate of Cryptosporidium oocysts, Giardia cysts, and microbial indicators during wastewater treatment and anaerobic sludge digestion. Canadian Journal of Microbiology, 45, 257-262.

CHEEMA, M. A., MALIK, M. A., HUSSAIN, A., SHAH, S. H. & BASRA, S. M. A. (2001) Effect of time and rate of nitrogen and phosphorus application on the growth and the seed and oil yields of canola (Brassica napus L.). J. Agron.Crop Sci, 186, 103-110.

CHEN, B., SHAN, X.-Q. & QIAN, J. (1996) Bioavailability index for quantitative evaluation of plant availability of extractable soil trace elements. Plant and Soil, 186, 275-283.

CHEN, C., JACKSON, G., NEIL, K., WICHMAN, D., JOHNSON, G. & JOHNSON, D. (2005) Determining the Feasibility of Early Seeding Canola in the Northern Great Plains. Agron. J., 97, 1252–1262.

CHLOPECKA, A. (1996a) Forms of Cd, Cu, Pb, and Zn in soil and their uptake by cereal crops when applied jointly as carbonates. Water, Air, and Soil Pollution, 87, 297-309.

CHLOPECKA, A. (1996b) Assessment of form of Cd, Zn and Pb in contaminated calcareous and gleyed soils in Southwest Poland. Science of the Total Environment, 188, 253-262.

CHLOPECKA, A. & ADRIANO, D. C. (1996) Mimicked In-Situ Stabilization of Metals in a Cropped Soil: Bioavailability and Chemical Form of Zinc. Environmental Science and Technology, 30, 3294-3303.

CHRISTENSEN, T. H. & TJELL, J. C. (1984) Interpretation of experimental results on cadmium crop uptake from sewage sludge amended soil. Dep. Environ. Eng.,Tech. Univ. Denmark, Lyngby,Den.

CIAVATTA, C., GOVI, M., SIMONI, A. & SEQUI, P. (1993) Evaluation of heavymetals during stabilization of organic matter in compost producedwithmunicipalwastes. Bioresour Technol ;43:147–53.

CIMINO, G. & TOSCANO, G. (1993) Effects of digested sewage sludge on yield and heavy metal accumulation in horticultural species. Bioresource Technology, 46, 217-20.

CLARKSON, D. T. & HANSON, J. B. (1980) The mineral nutrition of higher plants. Annual Review of Plant Physiology, 31, 239-298.

CLAYTON, P. M. & TILLER, K. G. (1979) A chemical method for determination of the heavy metal content of soils in environmental studies. Division of Soils Technical Paper (Australia, Commonwealth Scientific and Industrial Research Organization), 41, pp 17.

CLIFTON, P. (1999) Nutritional aspects of a novel oil. Proceedings of the 10th International Rapeseed Congress, Canberra, September 26-29, 1999. (Eds. N. Wratten & P. Salibury). (http:// www.regional.org.au/au/gcirc/).

COLTON, B. & POTTER, T. (1999) History. In: Canola in Australia: the First Thirty Years. Eds. P.A. Salisbury, T.D. Potter, G. McDonald and A.G. Green,Organising Committee of the 10th International Rapeseed Congress, Canberra, A.C.T., p. 1-4.

COLTON, R. T. & SYKES, J. D. (1992) Canola, Orange, NSW,Agfact P5.2.1., New South Wales Agriculture.

CORREA, R. S., WHITE, R. E. & WEATHERLEY, A. J. (2006) Risk of nitrate leaching from two soils amended with biosolids. Water Resources, 33, 453-462.

COURTNEY, R. G. & MULLEN, G. J. (2008) Soil quality and barley growth as influenced by the land application of two compost types. Bioresource Technology, 99, 2913-2918.

CUNNINGHAM, J. D., KEENEY, D. R. & RYAN, J. A. (1975) Phytotoxicity and uptake of metals added to soils as inorganic salts or in sewage sludge. Journal of Environmental Quality, 4, 460-462.

CURTIS, L. & LAWLEY, R. (2003) Micro-Facts: The Working Companion for Food Microbiologists, Fifth Edition.

DAHAB, M. F. & SURAMPALLI, R. Y. (2002) Effects of aerobic and anaerobic digestion systems on pathogen and pathogen indicator reduction in municipal sludge Water science and technology a journal of the International Association on Water Pollution Research, 46, 181-7.

DAHLIN, S. & WITTER, E. (1993) Effects of acidification and repeated sewage sludge application on C utilization by soil microorganisms. In: Eijsackers HJP, Hamers T, editors. Integrated Soil and Sediment Research: A Basis for Proper Protection., Kluwer Academic Publishers, Boston, USA.

DAHLIN S, W. E. (1993) Effects of acidification and repeated sewage sludge application on C utilization by soil microorganisms: In. IN EIJSACKERS HJP, H. T. & (Eds.) Integrated Soil and Sediment Research: A Basis for Proper Protection. Boston, USA, Academic Publishers.

DARMODY, R. G., FOSS, J. E., MCINTOSH, M. & WOLF, D. C. (1983) Municipal sewage sludge compost-amended soils: some spatiotemporal treatment effects. Journal of Environmental Quality, 12, 231-6.

DE VRIES, M. P. C. & TILLER, K. G. (1978) Sewage sludge as a soil amendment, with special reference to cadmium, copper, manganese, nickel, lead, and zinc - comparison of results from experiments conducted inside and outside a glasshouse. Environmental Pollution (1970-1979), 16, 231-40.

DEPARTMENT OF INDUSTRY WATER AND ENVIRONMENT (1999) Tasmanian Biosolids Reuse Guidelines. Hobart, TAS, Australia.

DEPORTES, I., BENOIT-GUYOD, J.-L. & ZMIROU, D. (1995) Hazard to man and the environment posed by the use of urban waste compost: a review. Science of the Total Environment, 172, 197-222.
DETROIT, M. I. & PELL, N. (1997) Manures and microbes: Public health and animal health problem? . J. Dairy Sci. , 89, 2673-2681.

DETURK, E. E. (1935) Adaptability of sewage sludge as a fertilizer. Sewage Works Journal, 7, 597-609.

DIEGOR, W., LONGERICH, H., ABRAJANO, T. & HORN, I. (2001) Applicability of a high pressure digestion technique to the analysis of sediment and soil samples by inductively coupled plasma-mass spectrometry. Analytica Chimica Acta, 431, 195-207.

DOLL, E. C. (1964) Lime for Michigan soils. Michigan Agricultural experiment station extension Bulletin 471.

DOLL, J. P. & ORAZEM, F. (1984) Production Economics: , Theory with Applications 2nd ed.

DOWDY, R. H. & HAM, G. E. (1977) Soybean growth and elemental content as influenced by soil amendments of sewage sludge and heavy metals: seedling studies. Agronomy Journal, 69, 300-303.

DOWDY, R. H., LARSON, W. E., TITRUD, J. M. & LATTERELL, J. J. (1978) Growth and metal uptake of snap beans grown on sewage sludge-amended soil: a four-year field study. Journal of Environmental Quality, 7, 252-257.

DOWDY, R. H., LATTERELL, J. J., HINESLY, T. D., GROSSMAN, R. B. & SULLIVAN, D. L. (1991) Trace metal movement in an Aeric Ochraqualf following 14 years of annual sludge applications. Journal of Environmental Quality, 20, 119-123.

DOWDY, R. H. & VOLK, V. V. (1983) Movement of heavy metals in soils. SSSA Special Publication, 11, 229-240.

DOXIADIS, C. A. (1973) Ancient Greek settlements third report.

DSE (2003) Biosolids in Victoria: Report on Options for Beneficial Use of Biosolids, Department of Sustainability and Environment Melbourne.

DUANE, M. J., FACCHETTI, S. & PIGOZZI, G. (1996) Site characterization of polluted soils and comparison of screening techniques for heavy metals by mobile ICP-MS, GFAAS/ICP-AES (fixed laboratory) and EDXRF (fixed laboratory). Science of the Total Environment, 177, 195-214.

DUBINSKY, P., JURIS, P. & MONCOL, D. J. (2000) Environmental Protection Against the Spread of Pathogenic Agents of Diseases Through the Wastes of Animal Production in the Slovak Republic, Harlequin, Ltd., Kosice, Slovak Republic.

DURING, R.-A. & GATH, S. (2002) Utilization of municipal organic wastes in agriculture: Where do we stand, where will we go? Journal of Plant Nutrition and Soil Science, 165, 544-556.

DVORAK, P., TLUSTOS, P., SZAKOVA, J., CERNY, J. & BALIK, J. (2003) Distribution of soil fractions of zinc and its uptake by potatoes, maize, wheat and barley after soil amendment by sludge and inorganic Zn salt. Plant, Soil and Environment, 49, 203-212.

EAMENS, G. J., LAVIS, A. M. & ROSS, A. D. (1996) Survival of pathogenic indicator bacteria in biosolids applied to agricultural land. Biosolids research in NSW, organic Waste Recycling Richmond, NSWAgriculture.

EC (1986) Council Directive 86/278/EEC. Official journal of European.

EC (2001) Evaluation of Sludge Treatments for Pathogen Reduction , Luxembourg : Office for Official Publications of the European Communities.

EFROYMSON, R. A., SAMPLE, B. E. & SUTER, G. W. (2001) Uptake of inorganic chemicals from soil by plant leaves: regressions of field data. Environmental Toxicology and Chemistry, 20, 2561-257.

EIJSACKERS, H. J. P. & HAMERS, T. (1993) Integrated Soil and Sediment Research: A Basis for Proper Protection. [In: Soil Environ., 1993; 1].

ENTZ, H. M., PAMELA, J. D. R., ROBERTA, M. G. & SCOTT, D. D. (2004) Yield Physiology of a Semidwarf and Tall Oat Cultivar. Crop Science, 44, 2116–2122.

ENZWEILER, J. & VENDEMIATTO, M. A. (2004) Analysis of sediments and soils by X-ray fluorescence spectrometry using matrix corrections based on fundamental parameters. Geostandards and Geoanalytical Research, 28, 103-112.

EPA NSW (1995) A searchable environmental valuation database. NSW Environmental Protection Authority. Available at http://www.epa.nsw.gov.au/envalue, accessed June 2008.

EPA VIC. (2004) Guidelines for Environmental Management. Biosolids land application. Publication 943. Melbourne, VIC, Australia.

EPA VICTORIA (2004) Guidelines for Environmental Management. Biosolids land application. In EPA (Ed.). Melbourne, VIC, Australia.

EPA VICTORIA (2004) Guidelines for Environmental Management. Biosolids land application, Melbourne, VIC, Australia.

EPSTEIN, E. (1997) The Science of Composting.

EPSTEIN, E. (2002) Land Application of Sewage Sludge and Biosolids.

EPSTEIN, E. (1975) Effect of sewage sludge on some soil physical properties. *J. Environ. Qual.,* 4**,** 139-142.

EPSTEIN, E. & CHANEY, R. L. (1978) Land disposal of toxic substances and water-related problems. Journal - Water Pollution Control Federation, 50, 2037-42.

EPSTEIN, E., TAYLOR, J. M. & CHANEY, R. L. (1976) Effects of sewage sludge and sludge compost applied to soil on some soil physical and chemical properties. Journal of Environmental Quality, 5, 422-426.

ERHART, E., HARTL, W. & PUTZ., B. (2005) Biowaste compost affects yield, nitrogen supply during the vegetation period and crop quality of agricultural crops ***Europ. J.*** *Agronomy,* 23**,** 305-314.

ERICH, M. S., FITZGERALD, C. B. & PORTER, G. A. (2002) The effect of organic amendments on phosphorus chemistry in a potato cropping system. Agriculture, Ecosystems & Environment, 88, 79-88.

ERIKSEN, G. N., COALE, F. J. & BOLLERO, G. A. (1999) Soil Nitrogen Dynamics and Maize Production in Municipal Solid Waste Amended Soil. Agronomy Journal, 91, 1009-1016.

ESTRADA, I. B., ALLER, A., ALLER, F., GOMEZ, X. & MORAN, A. (2004) The survival of Escherichia coli, faecal coliforms and enterobacteriaceae in general in soil treated with sludge from wastewater treatment plants. Bioresource Technology, 93, 191-198.

EUROPEAN COMMISSION (1986). Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture European Commission, Brussels.

EUROPEAN COMMISSION (2001) LANGENKAMP, H. & PART, P. (Eds.) Organic Contaminants in Sewage Sludge For Agricultural Use, European Commission Joint Research Centre Institute for Environment and Sustainability Soil and Waste Unit.

EVANYLO, G., SUKKARIYAH, B., EBORALL, M. A. & ZELAZNY, L. (2006) Bioavailability of heavy metals in biosolids-amended soil. Communications in Soil Science and Plant Analysis, 37, 2157-2170.

FARRELL, J. B., BHIDE, V. & SMITH, J. E. J. (1996) Development of EPA's new methods to quantify vector attraction of wastewater sludges. Water Environment Research, 68, 286-294.

FAULKNER DAVID (2001) Applying Biosolids: Issues for Virginia Agriculture USDA/Natural Resources, Conservation Service.

FEACHEM, R. G., BRADLEY, D. J., GARELICK, D. J. & MARA, D. D. (1983) Sanitation and Disease: Health Aspects of Excreta and Wastewater Management, John Wiley and Sons, New York.

FINE, P. & MINGELGRIN, U. (1996) Release of phosphorus from waste-activated sludge. Soil Science Society of America Journal, 60**,** 505-511.

FOLLE, F., SHUFORD, J. W., TAYLOR, R. W., MEHADI, A. A. & TADESSE, W. (1995) Effect of sludge treatment, heavy metls, phosphate rate, and pH on soil phosphorus. Communications in Soil Science and Plant Analysis, 26**,** 1369-1381.

FOUAD, A. & JUSTIN, V. D. Z. (2002) Biosolids from Waste to a Valuable Resource.

FRADKIN, L., GOYAL, S. M., BRUINS, R. J. F., GERBA, C. P., SCARPINO, P. & STARA, J. F. (1989) Municipal wastewater sludge: the potential public health impact of common pathogens. J Environ Health, 51, 148–152

FREEMAN, A. M. (1993) The Measurement of Environmental and Resources Values: Theory and Methods, Resources for the Future, Inc., Washington, DC.

FREEMAN, A. M. & HARRINGTON, W. (1990) Measuring welfare values of productivity changes. Southern Economic Journal 56, 892-904.

FRESQUEZ, P. R., FRANCIS, R. E. & DENNIS, G. L. (1990) Soil and vegetation responses to sewage sludge on a degraded semiarid broom snakeweed blue grama plant community. J. Range Manage., 43, 325–331.

FRICKE, K. & VOGTMANN, H. (1994) Compost quality – physical characteristics, nutrient content, heavy-metals and organic-chemicals. Toxicol Environ Chem 43:95–114.

FROSSARD, E., SINAJ, S. & DUFOUR, P. (1996) Phosphorus in urban sewage sludges as assessed by isotopic exchange. Soil Science Society of America Journal, 60, 179-82.

FROSSARD, E., SINAJ, S., ZHANG, L. M. & MOREL, J. L. (1996) The fate of sludge phosphorus in soil-plant systems. Soil Science Society of America Journal, 60, 1248-1253.

FUENTES, A., LLORENS, M., SAEZ, J., AGUILAR, M. I., ORTUNO, J. F. & MESEGUER, V. F. (2004) Phytotoxicity and heavy metals speciation of stabilized sewage sludges. Journal of Hazardous Materials, 108, 161-169.

FUKUSHI, K., BABEL, S. & BURAKRAI, S. (2003) Survival of Salmonella spp. in a simulated acid-phase anaerobic digester treating sewage sludge. Bioresource Technology, 86, 53-7.

GALE, A. J. (2007) The Australian Biosolids Partnership and Public Perceptions. Water Practice and Technology.

GANTZER, C., GASPARD, P., GALVEZ, L., HUYARD, A., DUMOUTHIER, N. & SCHWARTZBROD, J. (2001) Monitoring of bacterial and parasitological contamination during various treatment of sludge. Water Research, 35, 3763-3770.

GERARDI, H. M. (2002) Nitrification and Denitrification in the Activated Sludge Process, John Wiley & Sons, Inc.

GARCÍA, C., HERNÁNDEZ, T. & COSTA, F. (1990) The influence of composting and maturation processes on the heavy-metal extractability from some organic wastes. Biol Wastes 31:291–301.

GARIGLIO, N. F., BUYATTI, M. A., PILATTI, R. A., GONZALEZ ROSSIA, D. E. & ACOSTA, M. R. (2002) Use of a germination bioassay to test compost maturity of willow (Salix sp.) sawdust. New Zealand Journal of Crop and Horticultural Science, 30, 135-139.

GARREC, N., PICARD-BONNAUD, F. & POURCHER, A. M. (2003) Occurrence of Listeria sp. and L. monocytogenes in sewage sludge used for land application: effect of dewatering, liming and storage in tank on survival of Listeria species. FEMS Immunology and Medical Microbiology, 35, 275-283.

GARRIDO, S., DEL CAMPO, G. M., ESTELLER, M. V., VACA, R. & LUGO, J. (2005) Heavy metals in soil treated with sewage sludge composting, their effect on yield and uptake of broad bean seeds (Vicia faba L.). Water, Air, & Soil Pollution, 166, 303-319.

GASKIN, J. W., BROBST, R. B., MILLER, W. P. & TOLLNER, E. W. (2003) Long-term biosolids application effects on metal concentrations in soil and bermuda grass forage. Journal of Environmental Quality, 32, 146-152.

GENSTAT RELEASE 9 (2007) Lawes Agricultural Trust, Rothamsted, and Harpenden, UK.
GERALDSON, C. M., KLACAN, G. R. & LORENZ, O. A. (1973) Plant analysis as an aid in fertilizing vegetable crops. 365-379.

GERALDSON, C. M., KLACAN, G. R. & LORENZ, O. R. (1983) Analysis as an aid in fertilizing vegetable crops. In: ‘Soil Testing and Plant Analysis.’(Eds L.M. Welch and J.D. Beaton.). (Soil Sci. Soc.Amer. Inc.:Madison).

GERBA, C. P. (1983) Pathogens. In : Page, A.L., Gleason,T.L.I. , Smith, J.E.J. , Iskandar, I.K. and Sommers, L.E (Eds.), Utilization of Municipal Wastewater and Sludge on Land. University of California, Riverside.

GERBA, C. P. (1986) Human viruses in sediments, sludge and soils. In: R, C. (Ed.), Transport and Fate of Viruses in Soils: Field Studies. CRC Press, Boca Raton.

GERBA, P.C. & NWACHUKU, N. (2008) Occurrence and persistence of Escherichia coli O157:H7 in water

GERHARD, K., JON VAN, G. & JÜRGEN, K. (2005) The Biodiesel Handbook, AOCS Press, Champaign, Illinois, USA.

GERHARD, W. P. (1990) Sanitation and Sanitary Engineering, self-published, New York.

GERKE, J. & MEYER, U. (1995) Phosphate acquisition by red clover and black mustard on a humic podzol. Journal of Plant Nutrition, 18, 2409-2429.

GESSEL, P. D., HANSEN, N. C., GOYAL, S. M., JOHNSTON, L. J. & WEBB, J. (2004) Persistence of zoonotic pathogens in surface soil treated with different rates of liquid pig manure. Appl. Soil Ecol., 25, 237–243.

GIBBS, R., HU, C. J., HO, G. E., UNKOVICH, I. & PHILLIPS, P. (1994) Die-off of human pathogens in stored wastewater sludge and sludge applied to land. UWRAA Research Project no. [55-51(91/58)].

GIBBS, R. A., HU, C. J., HO, G. E. & UNKOVICH, I. (1997) Regrowth of fecal coliforms and salmonellae in stored biosolids and soil amended with biosolids. Water Science and Technology, 35, 269-275.

GIBSON, T., WHATMUFF, M., MICHALK, D. L. & EAMENS, G. (2002) New South Wales agriculture and Sydney water's biosolids research program, 1993-2000, implications for the NSW biosolids guidelines, in biosolids specialty conference I CD-Rom, ed., Australian Water Association, Sydney.

GILL, G. S. & HOLMES, J. E. (1997) Efficacy of cultural control methods for combating herbicide-resistant Lolium rigidum. Pesticide Science, 51 p. 352-358.

GOEPFERT, J. M. & HICKS, R. (1969) Effect of volatile fatty acids on Salmonella typhimurium. Journal of Bacteriology, 97, 956-8.

GOLUEKE, C. G. (1983) Epidemiological aspects of sludge handling and management, Part II. BioCycle, 24, 52–58.

GORING, C. A. I. & BARTHOLOMEW, W. V. (1952) Adsorption of mononucleotides, nucleic acids, and nucleoproteins by clays. Soil Science, 74, 149-64.

GOUGH, D. & FRASER, R. (1995) Long term planning for biosolids management in Sydney, in L.O.Kolarik and A.j. Priestley (eds.), Modern techniques in water and wastewater treatment. CSIRO, East Melbourne.

GREEN, I. D., MERRINGTON, G. & TIBBETT, M. (2003) Transfer of cadmium and zinc from sewage sludge amended soil through a plant-aphid system to newly emerged adult ladybirds (Coccinella septempunctata). Agric. Ecosyst. Environ. , 99 171-178.

GROTZ, N., FOX, T., CONNOLLY, E., PARK, W., GUERINOT, M. L. & EIDE, D. (1998) Identification of a family of zinc transporter genes from Arabidopsis that respond to zinc deficiency. Proceedings of the National Academy of Sciences of the United States of America, 95, 7220-7224.

GUAN, T. Y. & HOLLEY, R. A. (2003) Pathogen survival in swine manures environments and transmission of human enteric illness-a review. Journal of Environmental Quality, 32, 383-392.

GUERINOT, M. L. & EIDE, D. (1999) Zeroing in on zinc uptake in yeast and plants. Current Opinion in Plant Biology, 2, 244-249.

GUIXIN, P. U., MIKE, B., GLENN, B., PETER, W. & TERESA, C. (2004) Effects of Land Application of Two Biosolids on Plant Growth and Nitrogen Mineralization in a red ferrosol soil in subtropical Australia. New directions for a diverse planet. Proceedings of the 4th International Crop Science Brisbane, Australia.

GUO, X. W., FERNANDO, W. G. D. & ENTZ, M. (2005) Effects of crop rotation and tillage on blackleg disease of canola. Can J Plant Pathol. , 27, 53 – 57.

GUO, X. W., FERNANDO, W. G. D. & ENTZ, M. (2006) Dynamics of infection by Leptosphaeria maculans on canola (Brassica napus) as influenced by crop rotation and tillage. Archives of Phytopathology and Plant Protection, 1-10, PrEview article.

GUPTA, S. K. & ATEN, C. (1993) Comparison and evaluation of extraction media and their suitability in a simple model to predict the biological relevance of heavy metal concentrations in contaminated soils. International Journal of Environmental Analytical Chemistry, 51, 25-46.

HALL, G. E. M. (1993) Capabilities of production-oriented laboratories in water analysis using ICP-ES and ICP-MS. Journal of Geochemical Exploration, 49, 89-121.

HAMILL, M. L. (2002) The effect of cultivar, seeding date, seeding rate and nitrogen fertility on oat (Avena sativa L.) yield and milling quality. Winnipeg, Univ. of Manitoba, Canada.

HAMON, R. E. (1995) Identification of factors governing cadmium and zinc bioavailability in polluted soils, PhD Dissertation. University of Nottingham, UK.

.

HAMON, R. E., HOLM, P. E., LORENZ, S. E., MCGRATH, S. P. & CHRISTENSEN, T. H. (1999) Metal uptake by plants from sludge-amended soils: caution is required in the plateau interpretation. Plant and Soil, 216, 53-64.

HANS, D., BAUMANN, U., SOLLUK, K., RUETTIMANN, A. & UHLIG, S. (1992) Contaminated soil mapping (heavy metals). A comparison of atomic absorption spectrometry (AAS) and x-ray fluorescence analysis (XRF). Fresenius Environmental Bulletin, 1, 741-747.

HARRISON, R., DONGSEN, X., CHARLES, H. & DALE, W. C. (1994) Long-term effects of heavy applications of biosolids on organic matter and nutrient content of a coarse-textured forest soil Forest Ecology and Management, 66 65-177.

HARRISON, R. M. & CHIRGAWI, M. B. (1989) The assessment of air and soil as contributors of some trace metals to vegetable plants. II. Translocation of atmospheric and laboratory-generated cadmium aerosols to and within vegetable plants. Science of the Total Environment, 83, 35-45.

HARTL, W., PUTZ, B. & ERHART, E. (2003) Influence of rates and timing of biowaste compost application on rye yield and soil nitrate levels. European Journal of Soil Biology, 39, 129–139.

HASSEN, A., BELGUITH, K., JEDIDI, N., CHERIF, A., CHERIF, M. & BOUDABOUS, A. (2001). Microbial characterization during composting of municipal solid waste. Bioresource Technology, 80**,** 217-225.

HAUSLER, W. J., JR. (1999) Wastewater Microbiology, 2nd Edition. By Gabriel Bitton.

HAVLIN, J. L., BEATON, J. D., TISDALE, S. L. & NELSON, W. L. (1999) Soil Fertility and Fertilizers, Prentice Hall. Upper Saddle River, NJ. .

HAYGARTH, P. M., HARRISON, A. F. & JONES, K. C. (1995) Plant selenium from soil and the atmosphere. Journal of Environmental Quality, 24, 768-71.

HAYNES, R. J., DOMINY, C. S. & GRAHAM, M. H. (2003) Effect of agricultural land use on soil organic matter status and the composition of earthworm communities in KwaZulu-Natal, South Africa. Agriculture, Ecosystems and Environment, 95, 453-464.

HE, Q. B. & SINGH, B. R. (1993) Effect of organic matter on the distribution, extractability and uptake of Cd in soils. *J. Soil Sci.,* 44**,** 641-650.

HE, X. T., TRAINA, S. J. & LOGAN, T. J. (1992) Chemical properties of municipal solid waste composts. Journal of Environmental Quality, 21, 318-29.

HEANEY, D. J., MCGILL, W. B. & NGUYEN, C. (1988) Soil Test Quality Assurance Program, Final Report. Alberta Institute of Pedology, Edmonton, Alberta, Canada.

HEEMSBERGEN, D. A., WARNE, M. S. J., BROOS, K., BELL, M., NASH, D., MCLAUGHLIN, M., WHATMUFF, M., BARRY, G., PRITCHARD, D. & PENNEY, N. 2009. Application of phytotoxicity data to a new Australian soil quality guideline framework for biosolids. *Science of the Total Environment,* 407**,** 2546-2556.

HELYAR, K. R. (1976) Nitrogen cycling and soil acidification. Journal of the Australian Institute of Agricultural Science, 42, 217-21.
HENRY, D. P., FROST, A. J., O'BOYLE, D. & CAMERON, R. D. (1995) The isolation of Salmonellas from piggery waste water after orthodox pondage treatment. Australian veterinary journal, 72, 478-9.

HERNANDEZ, T., MORENO, J. I. & COSTA, F. (1991) Influence of sewage sludge application on crop yields and heavy metal availability. Soil Sci. Plant Nutr., 37, 201–210.

HERNANDEZ, T., MORENO, J. L., GARCıA, C. & PASCUAL, J. (1996) Transference of heavy metals from a calcareous soil amended with sewage-sludge compost to barley plants. Bioresource Technology, 55 251–258.

HEWITT, E. J. (1966) Sand and Water Culture Methods Used in the Study of Plant Nutrition. 2nd ed.

HIJNEN, W. A. M., WILLEMSEN-ZWAAGSTRA, J., HIEMSTRA, P., MEDEMA, G. J. & VAN DER KOOIJ, D. (2000) Removal of sulfite-reducing Clostridia spores by full-scale water treatment processes as a surrogate for protozoan (oo)cysts removal. Water Science and Technology, 41, 165-171.

HINESLY, T. D., BRAIDS, O. C., DICK, R. I., JONES, R. L. & MOLINA, J. A. E. (1994) Agricultural Benefits and Environmental Changes Resulting from the Use of Digested Sludge on Field Crops. USEPA: Washington, DC, EPA/530/SW-30.

HINESLY, T. D., JONES, R. L. & ZIEGLER, E. L. (1972) Effects on corn by applications of heated anaerobically digested sludge. Compost Science, 13, 26-30.

HINESLY, T. D., HANSEN, L. C. & DOTSON, G. K. (1984b.) Effects of using sewage sludge on agricultural and disturbed lands. EPA-600/S2-83-113. Order No. PB 84-117 142 Nat. Tech. Info. Service, Springfield, Virginia

HO, G. E., SIDHU, J. & GIBBS, R. A. (2000) Composting of wastewater biosolids Proceedings of international Workshop on Biosolids Management and Utilization, p.141–164

HOCKING, P., NORTON, R. & GOOD, T. (1999) Crop Nutrition. In : Salisbury, P.A., Potter, T.D., McDonald, G and Green A.G. (Eds). Canola in Australia, the first thirty years. Organising committee of the 10th International Rapeseed Congress, p. 15-23.

HOCKING, P. J. (1987) Effects of relief or onset of nitrogen stress at different stages on yield components of rapeseed. Proc. 6th Aust. Rapeseed Breeders and Agronomists Workshop. Canberra, ACT.

HOCKING, P. J. (2001) Effects of sowing time on nitrate and total nitrogen concentrations in field-grown canola (Brassica napus L.) and implications for plant analysis. Journal of Plant Nutrition, 24, 43-59.

HOCKING, P. J., PINKERTON, A. & GOOD, A. (1996) Recovery of field-grown canola from sulfur deficiency. Australian Journal of Experimental Agriculture, 36, 79-85.

HOCKING, P. J. & RANDALL, P. J. (1997) The response of dry land canola to nitrogen fertilizer: partitioning and mobilization of dry matter and nitrogen, and nitrogen effects on yield components. Field Crops Research, 54, 201-220.

HOCKING, P. J., RANDALL, P. J., DEMARKO, D. & BAMFORTH, I. (1997) Assessment of the nitrogen status field grown canola (Brassica napus) by plant analysis. Australian Journal of Experimental Agriculture, 37, 83-92.

HOERMAN, A., RIMHANEN-FINNE, R., MAUNULA, L., VON BONSDORFF, C.-H., TORVELA, N., HEIKINHEIMO, A. & HAENNINEN, M.-L. (2004) Campylobacter spp., Giardia spp., Cryptosporidium spp., noroviruses, and indicator organisms in surface water in Southwestern Finland, 2000-2001. Applied and Environmental Microbiology, 70, 87-95.

HOFFLAND, E. (1992) Quantitative evaluation of the role of organic acid exudation in the mobilization of rock phosphate by rape. Plant and Soil, 140, 279-289.

HOFFLAND, E., FINDENEGG, G. R. & NELEMANS, J. A. (1989) Solubilization of rock phosphate by rape. II. Local root exudation of organic acids as a response to phosphorus starvation. Plant and Soil, 113, 161-165.

HOLMES, M. R. J. (1980) Nutrition of the Oilseed Rape Crop.

HOODA, P.S., ALLOWAY, B.J., (1993b). The plant availability and DTPA extractability of trace metals in sludge-amended soils. Sci.T otal Environ. 149, 39–51.

HOODA, P.S., ALLOWAY, B.J., (1994). Sorption of Cd and Pb on selected temperate and semi-arid soils: effects of sludge application and ageing of sludged soils.W ater, Air Soil Pollut.74, 235–250.

HOODA, P. S. & ALLOWAY, B. J. (1994) Changes in operational fractions of trace metals in two soils during two-years of reaction time following sewage sludge treatment. International Journal of Environmental Analytical Chemistry, 57, 289-311.

HORAN, N. J., FLETCHER, L., BETMAL, S. M., WILKS, S. A. & KEEVIL, C. W. (2004). Die-off of enteric bacterial pathogens during mesophilic anaerobic digestion. *Water Research*, 38**,** 1113-1120.

HORST, W. J., KAMH, M., JIBRIN, J. M. & CHUDE, V. O. (2001) Agronomic measures for increasing P availability to crops. Plant and Soil, 237, 211-223.

HOVMAND, M. F., TJELL, J. C. & MOSBAEK, H. (1983) Plant uptake of airborne cadmium. Environmental Pollution, Series A Ecological and Biological, 30, 27-38.

HSEU, Z.-Y. & HUANG, C.-C. (2005) Nitrogen mineralization potentials in three tropical soils treated with biosolids. Chemosphere, 59, 447-454.

HUSSONG, D., BURGE, W. D. & ENKIRI, N. K. (1985) Occurrence, growth, and suppression of salmonellae in composted sewage sludge. Applied and Environmental Microbiology, 50, 887-893.

HYUN, H.-N., CHANG, A. C., PARKER, D. R. & PAGE, A. L. (1998) Cadmium solubility and phytoavailability in sludge-treated soil: effects of soil organic carbon. Journal of Environmental Quality, 27, 329-334.

IAEA (2004) Soil sampling for environmental contaminants, International Atomic Energy Agency. Vienna, Austria.

IBRAHIM, A. F., ABUSTEIT, E. O. & EL-METWALLY, E. M. A. (1989) Response of rapeseed (Brassica napus L.) growth, yield, oil content and its fatty acids to nitrogen rates and application times. Journal of Agronomy and Crop Science, 162, 107-112.

IMAS, P., BAR-YOSEF, B., KAFKAFI, U. & GANMORE-NEUMANN, R. (1997) Phosphate induced carboxylate and proton release by tomato roots. Plant and Soil, 191, 35-39.

IMPELLITTERI, C. A., SAXE, J. K., COCHRAN, M., JANSSEN, G. M. C. M. & ALLEN, H. E. (2003) Predicting the bioavailability of copper and zinc in soils: Modeling the partitioning of potentially bioavailable copper and zinc from soil solid to soil solution. Environ. Toxicol. Chem., 22, 1380-1386.

ISKANDAR, I. K. & ADRIANO, D. C. (1997) Remediation of soils contaminated with metals - a review of current practices in the U.S.A. In : Remediation of Soils Contaminated with Metals, I.K. Iskandar and D.C. Adriano, Eds., Science Reviews, Northwood.

IYENGAR, S. S., MARTENS, D. C. & MILLER, W. P. (1981) Distribution and plant availability of soil zinc fractions. Soil Science Society of America Journal, 45, 735-739.

JACKSON, G. D. (2000) Effects of nitrogen and sulfur on canola yield and nutrient uptake. Agronomy Journal, 92, 644-649.

JENNE, E. A. (1968) Controls on manganese, iron, cobalt, nickel, copper, and zinc concentrations in soils and water. Significant role of hydrous manganese and iron oxides. Advances in Chemistry Series, No. 73, 337-87.

JANZEN, H. H. 2004. Carbon cycling in earth systems--a soil science perspective. *Agriculture, Ecosystems & Environment*, 104**,** 399-417.

JEWELL, W. J. & SEABROOK, B. L. (1979) A history of land application as a treatment alternative. U.S. Environmental Protection Agency,Technical Report EPA 430/9-79-012, Washington, D.C.

JEYAKUMAR, P., LOGANATHAN, P., SIVAKUMARAN, S., ANDERSON, W. & MCLAREN, G. (2008) Copper and zinc spiking of biosolids: effect of incubation period on metal fractionation and speciation and microbial activity. Environ. Chem., 5**,** 347-354.

JING, J. & LOGAN, T. J. (1992) Effects of sewage sludge cadmium concentration on chemical extractability and plant uptake. Journal of Environmental Quality, 21, 73-81.

JOANNA, M. H. & DAVID, B. W. (2004) Effects of surface-applied biosolids on grass seedling emergence in the Chihuahuan desert. Journal of Arid Environments, 58, 19–42.

JOHANSSON, C., KRON, E., SVENSSON, S. E., CARLSBÆK, M. & REEH, U. (1997) Compost quality and potential for use - Literature review and final report. AFR report 154 Swedish Environmental Protection Agency Stockholm.

JOHANSSON, M., STENBERG, B. & TORSTENSSON, L. (1999) Microbiological and chemical changes in two arable soils after long-term sludge amendments. Biology and Fertility of Soils, 30, 160-167.

JOKINEN, R. (1990) Effect of phosphorus precipitation chemicals on characteristics and agricultural value of municipal sewage sludges. Acta Agric. Scand, 40, 141–147.

JONES, A. A. (1982) X-ray fluorescence spectrometry. In: Page AL, Miller RH, Keeney DR, editors. Methods of soil analysis. Part 2: Chemical and microbiological properties. 2nd ed. Madison (WI): Agronomy 9.

JONES, A. A. (1982) X-ray fluorescence spectrometry. In: Page AL, Miller RH, Keeney DR, editors. Methods of soil analysis. Part 2: Chemical and microbiological properties. 2nd ed. Madison (WI).Agronomy 9
J. BENTON JONE, J. (2003) Agronomic Handbook: Management of Crops, Soils, and Their Fertility.

J. BENTON JONE, J. (2001) Laboratory Guide for Conducting Soil Tests and Plant Analysis.

JONES, P. W. (1986) Sewage sludge as a vector of salmonellosis. In: Block, J.C., Haielaar, A.H., L'Hermite, P. (Eds.), Epidemiological Studies of Risks Associated with the Agricultural use of Sewage Sludge. Elsevier, London.

JOSHUA, W. D., MICHALK, D. L., CURTIS, I. H., SALT, M. & OSBORNE, G. J. (1998) The potential for contamination of soil and surface waters from sewage sludge (biosolids) in a sheep grazing study, Australia. Geoderma, 84, 135–156.

JURADO, P. & WESTER, D. B. (2001) Effects of biosolids on tobosagrass growth in the Chihuahuan desert. Journal of Range Management 54, 89-95.

JUST, R. E., HUETH, L. D. & SCHMITZ, A. (2004) The Welfare Economics of Public Policy, Edward Elgar. Northampton, MA.

KABATA-PENDIAS, A. & PENDIAS, H. (1989) Trace Elements in Soils and Plants.

KALRA, Y. P. & EDITOR (1998) Handbook of Reference Methods for Plant Analysis.

KARACA, A. (2004) Effect of organic wastes on the extractability of cadmium, copper, nickel, and zinc in soil. Geoderma, 122, 297-303.

KARAPANAGIOTIS, N.K., STERRITT, R.M., LESTER, J.N., 1991. Heavy metal complexation in sludge-amended soil.The role of organic matter in metal retention. Environ.T echnol.12, 1107–1116.

KARATHANASIS, A. D. & HAJEK, B. F. (1996) Elemental Analysis by X-Ray Fluorescence Spectroscopy. In: Methods of Soil analyses: chemical methods part 3. Soil Science Society of America Book Series Number 5. American Society of Agronomy, Madison, WI.

KAREN, D. L., DUFFY, M. D. & COLVIN, T. S. (1995) Nutrient, labor, energy, and economic evaluations of two farming systems in Iowa. J. Prod. Agric., 8, 540–546.

KEENEY, D. R. & NELSON, D. W. (1982) Nitrogen in organic forms. In : A.L. Page, R.H. Miller, and D.R. Keeney, Eds. Methods of Soil Analysis. Part 2. Agronomy No. 9. American Society of Agronomy, Madison, WI.

KELLING, K. A., WALSH, L. M., KEENEY, D. R., RYAN, J. A. & PETERSON, A. E. (1977) A field study of the agricultural use of sewage sludge: II. Effect on soil nitrogen and phosphorus. Journal of Environmental Quality, 6, 345-352.

KENNETH, E. M. & E.B., N. (2005) Valuing the environment as a factor of production. In: Handbook of Environmental Economics, Volume 2 Valuing Environmental Changes, Edited by K.-G. Mäler and J.R. Vincent. Department of Agricultural and Resource Economics. University of Maryland.

KIDD, P. S., DOMINGUEZ-RODRIGUEZ, M. J., DIEZ, J. & MONTERROSO, C. (2007) Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. Chemosphere, 66, 1458-1467.

KIEKENS, L., COTTENIE, A. & VAN LANDSCHOOT, G. (1984) Chemical activity and biological effect of sludge-borne heavy metals and inorganic metal salts added to soils. Plant and Soil, 79, 89-99.

KIM, B., MCBRIDE, M. B., RICHARDS, B. K. & STEENHUIS, T. S. (2007) The long-term effect of sludge application on Cu, Zn, and Mo behavior in soils and accumulation in soybean seeds. Plant and Soil, 299, 227-236.

KING, L. D. (1988) Effect of selected soil properties on cadmium content of tobacco. Journal of Environmental Quality, 17, 251-255.

KING, L. D. & HAJJAR, L. M. (1990) The residual effect of sewage sludge on heavy metal content of tobacco and peanut. Journal of Environmental Quality, 19, 738-748.

KIRCHMANN, H., PICHLMAYER, F. & GERZABEK, M. H. (1996) Sulfur balances and sulfur-34 abundance in a long-term fertilizer experiment. Soil Science Society of America Journal, 60, 174-178.

KIRKEGAARD, J. A., GARDNER, P. A., ANGUS, J. F. & KOETZ, E. (1994) Effect of Brassica break crops on the growth and yield of wheat. Australian Journal of Agricultural Research, 45, 529-545.

KIRKEGAARD, J. A., MELE, P. M. & HOWE, G. N. (1999) Enhanced accumulation of mineral-N following canola. *Australian Journal of Experimental Agriculture* 39**,** 587 - 593.

KIRKEGAARD, J. A., SARWAR, M., WONG, P. T. W. & MEAD, A. (1998) Biofumigation by brassicas reduces Take-all infection. Proceedings of 9th Australian Agronomy Conference, Wagga Wagga, July 1998, February 2001. (Ed. D.L.Michalk and J.E. Pratley), pp.465-468.

KIRKEGAARD, J. A., SARWAR, M., WONG, P. T. W., MEAD, A., HOWE, G. & NEWELL, M. (2000) Field studies on the biofumigation of take-all by Brassica crops. Australian Journal of Agricultural Research, 51 445-456.

KIRKHAM, M. B. (1982) Agricultural use of phosphorus in sewage sludges. Adv. Agron. , 35, 129–163.

KHARBANDA, P. (1999) Influence of tillage on blackleg and other diseases of canola in rotation with barley [online] Available from http://www.scdc.sk.ca/php-bin/articles/a038.html.

KNIGHT, B. P., CHAUDRI, A. M., MCGRATH, S. P. & GILLER, K. E. (1998) Determination of chemical availability of cadmium and zinc in soils using inert soil moisture samplers. Environmental Pollution, 99, 293-298.

KNOX, A. S., GAMERDINGER, A. P., ADRIANO, D. C., KOLKA, R. K. & KAPLAN, D. I. Sources and practices contributing to soil contamination, in Bioremediation of Contaminated Soils, Am. Soc. Agron., Madison, WI, 1999, 53.

KOCHIAN, L. V., SHAFF, J. E. & RYAN, P. R. (1991) Microelectrode-based investigations into the relationship between Al toxicity and root-cell membrane transport processes. Current Topics in Plant Biochem Physiol 10, 117-133.

KONDRA, Z. P., CAMPBELL, D. C. & KINK, J. R. (1983) Temperature effects on germination of rapeseed (Brassica napus L. and Brassica campestris L.). Can.Journal of Plant Science, 63, 1063-1065.

KORCAK, R. F. & FANNING, D. S. (1985) Availability of applied heavy metals as a function of type of soil material and metal source. Soil Science, 140, 23-34.

KOWAL, N. E. (1985) Health effects of Land Application of Municipal Sludge, EPA/600/1-85/015. U.S. EPA, Cincinnati. Ohio.

KUDVA, I. T., BLANCH, K. & HOVDE, C. J. (1998) Analysis of Escherichia coli O157:H7 survival in ovine or bovine manure and manure slurry. Applied and Environmental Microbiology, 64, 3166-3174.

KUNTE, D. P., YEOLE, T. Y., CHIPLONKAR, S. A. & RANADE, D. R. (1998) Inactivation of Salmonella typhi by high levels of volatile fatty acids during anaerobic digestion. Journal of Applied Microbiology, 84, 138-142.

KUO, S., JELLUM, E. J. & BAKER, A. S. 1985. Effects of soil type, liming, and sludge application on zinc and cadmium availability to Swiss chard. *Soil Science,* 139**,** 122-130.

KYLE, M. & MCCLINTOCK, S. A. (1995) The availability of phosphorus in municipal wastewater sludge as a function of the phosphorus removal process and sludge treatment method. Water Environment Research, 67, 282-289.

LAKE, D.L., KIRK, P.W.W., LESTER, J.N., (1984). Fractionation, characterization, and speciation of heavy metals in sewage sludge and sludge-amended soils: a review.J. Environ. Qual.13, 175–183.

LANCE, J. C. (1977) Fate of pathogens in saturated and unsaturated soils. In: National Conference on Composting of Municipal Residues and Sludges. Silver Spring, Maryland. Information Transfer, Inc., Rockville, Maryland, and Hazardous Materials Control Research Institute.

LANE, R. A. (1988) The effect of sewage sludge application to bermuda grass on forage quality production, and metal accumulation. Agriculture, Ecosystems & Environment, 20, 209-219.

LANG, X., DALAI, A. K., BAKHSHI, N. N., REANEY, M. J. & HERTZ, P. B. (2001) Preparation and characterization of bio-diesels from various bio-oils. *Bioresource Technology,* 80**,** 53-62.

LASOBRAS, J., DELLUNDE, J., JOFRE, J. & LUCENA, F. (1999) Occurrence and levels of phages proposed as surrogate indicators of enteric viruses in different types of sludges. Journal of Applied Microbiology, 86, 723-729.

LAVADO, R. S., RODRIGUEZ, M. B. & TABOADA, M. A. (2005) Treatment with biosolids affects soil availability and plant uptake of potentially toxic elements. Agriculture, Ecosystems & Environment, 109, 360-364.

LEBLANC, J. R., MATTHEWS, P. & RICHARD, P. R. (2008) Global atlas of excreta, wastewater sludge, and biosolids management: moving forward the sustainable and welcome uses of a global resource. United Nations Human Settlements Programme (UN-HABITAT).

LECLERC, B., GEORGES, P., CAUWEL, B. & LAIRON, D. (1995) A five year study on nitrate leaching under crops fertilised with mineral and organic fertilisers in lysimeters. Biol. Agriculture Horticulture, 11, 301–308.

LEE, K. W. & KEENEY, D. R. (1975) Cadmium and zinc additions to Wisconsin soils by commercial fertilizers and waste water sludge application. Water, Air, and Soil Pollution, 5, 109-112.

LEIFELD, J., SIEBERT, S. & KOGEL-KNABNER, I. (2002) Biological activity and organic matter mineralization of soils amended with biowaste composts. Journal of Plant Nutrition and Soil Science, 165, 151-159.

LEWIS, D. L. & GATTIE, D. K. (2002) Pathogen risks from applying sewage sludge to land. Environmental Science and Technology, 36, 286A-293A.

LI, Z., RYAN, J.A., CHEN, J.L., AL-ABED, S.R., (2001). Adsorption of cadmium on biosolids-amended soils.J.Envir on. Qual. 30, 903–911.

LI, J. & WU, W. Z. (2003) Analysis of sludge produce, deal with and disposal in domestic and foreign land. International Workshop Corpus of Sludge Deal with and Disposal Technology and Equipment, p. 1–11.

LINDSAY, W. L. (1979) Chemical Equilibria in Soils.

LIVINGSTON, N. J. & JONG, E. D. (1990) Matric and osmotic potential effects on seedling emergence at different temperatures. Agron. J., 82, 995–998.

LOGAN, T. J. & CHANEY, R. L. (1983) Utilization of municipal wastewater and sludges on land-metals, in Workshop on Utilization of Municipal Wastewater and Sludge on Land, Denver, CO.

LONGERICH, H. P. (1995) Analysis of pressed pellets of geological samples using wavelength-dispersive x-ray fluorescence spectrometry. X-Ray Spectrometry, 24, 123-136.

LUEHS, W. (1996) Brassica Oilseeds. Production and Utilization. Edited by D. Kimber and D. I. McGregor.

LUO, Y. M. & CHRISTIE, P. (1998) Bioavailability of copper and zinc in soils treated with alkaline stabilized sewage sludges. Journal of Environmental Quality, 27, 335-342.

MA, G. & LI, G. (1989) Application of x-ray fluorescence spectrometry to the analysis of geochemical prospecting samples in China. X-Ray Spectrometry, 18, 199-205.

MACLEAN, K. S., ROBINSON, A. R. & MACCONNELL, H. M. (1987) The effect of sewage-sludge on the heavy metal content of soils and plant tissue. Communications in Soil Science and Plant Analysis, 18, 1303-16.

MADEJON, E., BURGOS, P., LOPEZ, R. & CABRERA, F. (2003) Agricultural use of three organic residues: effect on orange production and on properties of a soil of the Comarca Costa de Huelva' (SW Spain). Nutrient Cycling in Agroecosystems, 65, 281-288.

MAEKINEN, E., KORHONEN, M., VISKARI, E.-L., HAAPAMAEKI, S., JAERVINEN, M. & LU, L. (2006) Comparison of XRF and FAAS Methods in Analyzing CCA Contaminated Soils. Water, Air, & Soil Pollution, 171, 95-110.

MAFTOUN, M, MOSHIRI, F., KARIMIAN, N. & RONAGHI, A.M. (2004) Effects of two organic wastes in combination with phosphorus on growth and chemical composition of spinach and soil properties. J Plant Nutr 27:1635–51.

MAGUIRE, R. O., SIMS, J. T. & COALE, F. J. (2000) Phosphorus fractionation in biosolids-amended soils: relationship to soluble and desorbable phosphorus. Soil Science Society of America Journal, 64, 2018-2024.

MAGUIRE, R. O., SIMS, J. T. & COALE, F. J. (2000) Phosphorus solubility in biosolids-amended farm soils in the Mid-Atlantic region of the USA. Journal of Environmental Quality, 29, 1225-1233.

MAGUIRE, R. O., SIMS, J. T., DENTEL, S. K., COALE, F. J. & MAH, J. T. (2001) Relationships between biosolids treatment process and soil phosphorus availability. Journal of Environmental Quality, 30, 1023-1033.

MAHLER, R. J., BINGHAM, F. T. & PAGE, A. L. (1978) Cadmium-enriched sewage sludge application to acid and calcareous soils: Effect on yield and cadmium uptake by lettuce and chard. Journal of Environmental Quality, 7, p274-281.

MAHLER, R.J., RYAN, J.A., REED, T., (1987). Cadmium sulphate application to sludge-amended soils I.Effect on yield and cadmium availability to plants.Sci. Total Environ. 67, 117–131.

MAILER, R. J., COLTON, R. T. & O'BREE, B. L. (1998) Quality of Australian Canola. Canola Association of Australia. ISSN 1322-9397.

MALER, K.-G. (1992) Production function approach in developing countries. In: J.R. Vincent, E. W.Crawford, J. P. Hoehn, eds., Valuing Environmental Benefits in Developing Countries Special report 29, Michigan State University.

MAMO, M., ROSEN, C. J. & HALBACH, T. R. (1999) Nitrogen availability and leaching from soil amended with municipal solid waste compost. Journal of Environmental Quality, 28, 1074-1078.

MANTOVI, P., BALDONI, G. & TODERI, G. (2005) Reuse of liquid, dewatered, and composted sewage sludge on agricultural land: effects of long-term application on soil and crop. Water Research, 39, 289-296.

MARCHIOL, L., ASSOLARI, S., SACCO, P. & ZERBI, G. 2004. Phytoextraction of heavy metals by canola (Brassica napus) and radish (Raphanus sativus) grown on multicontaminated soil. Environmental Pollution, 132**,** 21-27.

MARKANDYA, A., PATRICE, H., BELLU, L. G., VITO, C. & ELGAR, E. (2000) Environmental Economics for Sustainable Growth, (A Handbook for Practitioners).

MAROONDAH, A. P. S. (2001) A Guide to the Indigenous Plants of the Greater Melbourne Area, Flora of Melbourne Hyland House, Melbourne.

MARSHALL, H. G., KOLB, F. L. & ROTH., G. W. (1987) Effects of nitrogen fertilizer rate, seeding rate, and row spacing on semi-dwarf and conventional height spring oat. Crop Science, 27, 572–575.

MARTIN, J. H., JR., BOSTIAN, H. E. & STERN, G. (1990) Reductions of enteric microorganisms during aerobic sludge digestion. Water Research, 24, 1377-85.

MARTINEZ, C.E., JACOBSON, A., MCBRIDE, M.B., (2001). Thermally induced changes in metal solubility of contaminated soils is linked to mineral recrystallization and organic matter transformations. Environ.Sci.T echnol.35, 908–916.

MARTINEZ, F., CUEVAS, C., TERESA, WALTER & INGRID, I. (2002) Urban organic wastes effects on soil chemical properties in degraded semiarid ecosystem. In: Seventeenth WCSS, Symposium No. 20, Thailand. pp.1–9.

MAWDSLEY, J. L., BARDGETT, R. D., MERRY, R. J., PAIN, B. F. & THEODORU, M. K. (1995) Pathogens in livestock waste, their potential for movement through soil and environmental pollution. Appl Soil Ecol 2, 1–15.

MC GRATH, S. P. (1984) Metal concentration in sludges and soil from a long-term field trial. J. Agric. Sci., Cambridge, 103, 25–35.

MC GRATH, S. P. (1987) Long-term studies of metal transfers following application of sewage sludge. In: Coughtrey, P.J, Martin, L.H., Unsworth, M.H. (Eds.), Pollutant transport and fate in ecosystems. Special Publ. No. 6 of the British Ecological Society. Blackwell Scientific, Oxford.

MCBRIDE, M. B. (1985) Sorption of copper(II) on aluminum hydroxide as affected by phosphate. Soil Science Society of America Journal, 49, 843-6.

MCBRIDE, M. B. (1989) Reactions controlling heavy metal solubility in soils. Advances in Soil Science, 10, 1-56.

MCBRIDE, M. B. (1995) Toxic metal accumulation from agricultural use of sludge: are USEPA regulations protective? Journal of Environmental Quality, 24, 5-18.

MCBRIDE, M. B. (1998) Soluble trace metals in alkaline stabilized sludge products. Journal of Environmental Quality, 27, 578-584.

MCBRIDE, M.B., RICHARDS, B.K., STEENHUIS, T., SPIERS, G., (1999).Long-term leaching of trace elements in a heavily sludge-amended silty clay loam soil.Soil Sci. 164, 613–623.

MCBRIDE, M. B. (2003) Toxic metals in sewage sludge-amended soils: has promotion of beneficial use discounted the risks? Advances in Environmental Research, 8, 5-19.

MCBRIDE, M. B. & CHERNEY, J. (2004) Molybdenum, sulfur, and other trace elements in farm soils and forages after sewage sludge application. Communications in Soil Science and Plant Analysis, 35, 517-535.

MCBRIDE, M. B., RICHARDS, B. K. & STEENHUIS, T. (2004) Bioavailability and crop uptake of trace elements in soil columns amended with sewage sludge products. Plant and Soil, 262, 71-84.

MCCONNELL, K. E., AND BOCKSTAEL, N.E. (2005) Valuing the environment as a factor of production, in : Handbook of Environmental Economics,Volume 2 Valuing Environmental changes edited by Karl-Goran Maler .Department of Agricultural and Resource Economics, University of Maryland.

MCCOY, J. L., SIKORA, J. & WEIL., R. R. (1986) Plant availability of phosphorus in sewage sludge. J. Environ. Qual., 15, 403–409.

MCFARLAND, M. J. (2001) Biosolids engineering. McGraw-Hill, Professional Engineering, New York. 800 p.

MCGRATHS, P. (1987) Long-term studies of metal transfer following applications of sewage sludge. In Pollutant Transport and Fate in Ecosystems (eds P.J. Coughtrey, M.H. Martin & M.H. Unsworth), pp. 301-317. Blackwell Scientific Publications, Oxford.

MCGRATH, S. P., CHANG, A. C., PAGE, A. L. & WITTER, E. (1994) Land application of sewage sludge: scientific perspectives of heavy metal loading limits in Europe and the United States. Environmental Reviews (Ottawa), 2, 108-118.

MCLAUGHLIN, M. J. (1984) Land application of sewage sludge: phosphorus considerations. South African J. Plant Soil, 1, 21–29.

MCLAUGHLIN, M. J., HAMON, R. E., MCLAREN, R. G., SPEIR, T. W. & ROGERS, S. L. (2000) Review: A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. Australian Journal of Soil Research, 38, 1037-1086.

MCLAUGHLIN, M.J., STEVENS, D.P., ZARCINAS, B.A., COOK, N. (2000). Testing soils and plants for heavy metals. Communications in Soil Science and Plant Analysis 31, 1661–1700.

MCLAUGHLIN, M. J., WHATMUFF, M., WARNE, M., HEEMSBERGEN, D., BARRY, G., BELL, M., NASH, D. & PRITCHARD, D. (2006) A Field Investigation of Solubility and Food Chain Accumulation of Biosolid-Cadmium Across Diverse Soil Types. Environmental Chemistry, 3, 428-432.

MELTON ENVIRONMENTAL ATLAS (DRAFT) (2007).

MELTON SHIRE COUNCIL NATURAL ENVIRONMENT (2004).

MENDOZA, J., GARRIDO, T., CASTILLO, G. & SAN MARTIN, N. (2006) Metal availability and uptake by sorghum plants grown in soils amended with sludge from different treatments. Chemosphere, 65, 2304-2312.

MENGEL, K. & KIRKBY, E. A. (1978) Principles of Plant Nutrition, p593.

MERMUT, A. R., JAIN, J. C., SONG, L., KERRICH, R., KOZAK, L. & JANA, S. (1996) Trace element concentrations of selected soils and fertilizers in Saskatchewan, Canada. Journal of Environmental Quality, 25, 845-853.

MERRINGTON, G., OLIVER, I., SMERNIK, R. J. & MCLAUGHLIN, M. J. 2003. The influence of sewage sludge properties on sludge-borne metal availability. Advances in Environmental Research, 8**,** 21-36.

MEYER, V. F., REDENTE, E. F., BARBARICK, K. A., BROBST, R. B., PASCHKE, M. W. & MILLER, A. L. (2004) Plant and soil responses to biosolids application following forest fire. Journal of Environmental Quality, 33, 873-881.

MISHRA, S. K. (2000) Valuation of Environmental Goods and Services, : an Institutionalistic Assessment.

MOCE-LLIVINA, L., MUNIESA, M., PIMENTA-VALE, H., LUCENA, F. & JOFRE, J. (2003) Survival of bacterial indicator species and bacteriophages after thermal treatment of sludge and sewage. Applied and Environmental Microbiology, 69, 1452-1456.

MOOLENAAR, S. W. & BELTRAMI, P. (1998) Heavy metal balances of an Italian soil as affected by sewage sludge and Bordeaux mixture applications. Journal of Environmental Quality, 27, 828-835.

MOORE, P. A. J., DANIEL, T. C. & EDWARDS, D. R. (1999) Reducing phosphorus runoff and improving poultry production with alum. Poultry science, 78, 692-698.

MORAL, R., MORENO-CASELLES, J., PEREZ-MURCIA, M. & PEREZ-ESPINOSA, A. (2002) Improving the micronutrient availability in calcareous soils by sewage sludge amendment. Communications in Soil Science and Plant Analysis, 33, 3015-3022.

MOREL, J. L. & GUCKERT, A. (1981) Influence of limed sludge on soil organic matter and soil physical properties. In: Catroux G, L'Hermite P, Suess E, editors. The Influence of Sewage Sludge Application on Physical and Biological Properties of Soils. London 7 D.Reidel Publishing

MORENO, J. L., GARCIA, C., HERNANDEZ, T. & PASCUAL, J. A. (1996) Transference of heavy metals from a calcareous soil amended with sewage-sludge compost to barley plants. Bioresource Technology, 55, 251-258.

MORERA, M. T., ECHEVERRIA, J. & GARRIDO, J. (2002) Bioavailability of heavy metals in soils amended with sewage sludge. Canadian Journal of Soil Science, 82, 433-438.

MORRISON, M. (2001) Non-market Valuation Databases: How Useful Are They?", Economic Analysis and Policy, 31[1]: 33-56.

MORRISON , M. J., MCVETTY, P. B. E. & SHAYKEWICH, C. F. (1989) The determination and verification of a baseline temperature for the growth of Westar summer rape. Can.Journal of Plant Science, 69, 455-464.

MORTVEDT, J. J., MAYS, D. A. & OSBORN, G. (1981) Uptake by wheat of cadmium and other heavy metal contaminants in phosphate fertilizers. Journal of Environmental Quality, 10, 193-197.

MOSQUERA-LOSADA, M. R., LOPEZ-DIAZ, L. & RIGUEIRO-RODRIGUEZ, A. (2001) Sewage sludge fertilisation of a silvopastoral system with pines in northwestern Spain. Agrofor Syst 53, 1–10.

MURILLO, J. M., CABRERA, F. & LOPEZ, R. (1997) Response of clover (Trifolium fragiferum L.) cv. Salina to a heavy urban compost application. Compost Science and Utilization, 5, 15-25.

MURILLO, J. M., CABRERA, F., LOPEZ, R. & MARTIN-OLMENDO, P. (1995) Testing low quality urban compost for agriculture: germination and seedling performance of plants. Agriculture, ecosystem and environment, 54, 127-135.

NATIONAL RESOURCE MANAGEMENT MINISTERS COUNCIL (2004) Guidelines for sewerage systems – Biosolids management. National Water Quality Management Strategy Paper 13. Canberra, ACT, Australia.

NBRP (2007) Draft position paper, Recommendations of the Australian National Biosolids Research Program on Biosolids Guidelines. *Biosolids Specialty III Conference.*

NBRP, (2007) Victoria Final Report - June 2007 Published by the Victorian Government Department of Primary Industries Primary Industries Research Victoria, Soil and Water Platform Hazeldean Rd, Ellinbank, Victoria 3821 Australia.

NEILSEN, G. H., HOGUE, E. J., NEILSEN, D. & ZEBARTH, B. J. (1998) Evaluation of organic wastes as soil amendments for cultivation of carrot and chard on irrigated sandy soils. Canadian Journal of Soil Science, 78, 217-225.

NELL, J. H., STEER, A. G. & VAN RENSBURG, P. A. J. (1983) Hygienic quality of sewage sludge compost. Water Sci Technol 15, 181–194

NEPC (1999) National Environment Protection (Assessment of Site Contamination) Measure: Schedule B (1) Guideline on the Investigation Levels for Soil and Groundwater. National Environment Protection Council, Adelaide, 16p.

NICHOLSON, F. A., GROVES, S. J. & CHAMBERS, B. J. (2005) Pathogen survival during livestock manure storage and following land application. Bioresource Technology, 96, 135-143.

NIEWOLAK, S. (1994) The sanitary and bacteriological estimation of surface and underground waters in the region of a pig slurry agricultural utilization. Pol. J. Environ. Stud., 3, 31–37.

NIEWOLAK, S. & SZELAGIEWICZ, M. (1997) Sanitary-bacteriological and parasitological estimation of sewage sludges from chosen sewage treatment plants on the Mazurian Lake District and utilization in agriculture. Pol. J. Environ. Stud., 6, 45– 51.

NOER, O. J. (1926) Activated sludge: Its production, composition, and value as a fertilizer. Journal of the American Society of Agronomy, 18, 962-963.

NORTON, R., KIRKEGAARD, J., ANGUS, J. & POTTER, T. (1999) Canola in rotation. the 10th International rapeseed congress. Canberra, Australia.

NORTON, R. M. (1993) The effects of applied nitrogen on the growth, yield, water use and quality of rapeseed (Brassica napus) in the Wimmera. LaTrobe University,Bundoora.

NORTON, R. M., KIRKEGAARD, J. A., ANGUS, J. F. & POTTER, T. D. (1999) Canola in rotations. In : Salisbury, P.A., Potter, T.D., McDonald, G and Green A.G. (Eds). Canola in Australia, the first thirty years. Organising committee of the 10th International Rapeseed Congress, pp. 23-28.

NSW EPA (1997) Environmental guidelines: Use and disposal of biosolids products. Sydney, New South Wales, Australia.

NSW EPA (1997) Environmental Guidelines: Use and Disposal of Biosolids Products, Chatswood, NSW.

NYKIFORUK, C. L. & JOHNSON-FLANAGAN, A. M. (1994) Germination and early seedling development under low temperature in canola. Crop Science, 34, 1047–1054.

O.I. ANALYTICAL (2001b) Ammonia in soil and plant extracts by segmented flow analysis (SFA), College Station, TX.

O.I. ANALYTICAL. (2001a) Nitrate plus nitrite nitrogen and nitrite nitrogen in soil and plant extracts by segmented flow analysis (SFA), College Station, TX.

O'CONNOR, G. A. & H.A., E. (2000) Co-application of biosolids and water treatment residualds. Final report. Florida Dep. Of Environ. Protection, Tallahassee.

O'CONNOR, G. A., SARKAR, D., BRINTON, S. R., ELLIOTT, H. A. & MARTIN, F. G. (2004) Phytoavailability of biosolids phosphorus. Journal of Environmental Quality, 33**,**703-712.

OBERLE, S. L. & KEENEY, R. D. (1994) Interactions of sewage sludge with soil–crop–water systems. In: Clapp CE, Larson WE, Dowdy RH, editors. Sewage sludge: Land Utilisation and the Environment. (SSSA Miscellaneous Publication). Madison7 Soil Science Society of America.

O'CONNER, G. A. (1988) Use and misuse of the DTPA soil test. Journal of Environmental Quality, 17, 715-718.

O'CONNOR, G. A., SARKAR, D., BRINTON, S. R., ELLIOTT, H. A. & G., M. F. (2004) Phytoavailability of biosolids phosphorus Journal of environmental quality 33**,** 703-712

OGDEN, I. D., HEPBURN, N. F., MACRAE, M., STRACHAN, N. J. C., FENLON, D. R., RUSBRIDGE, S. M. & PENNINGTON, T. H. (2002) Long-term survival of Escherichia coli O157 on pasture following an outbreak associated with sheep at a scout camp. Letters in applied microbiology, 34, 100-104.

OGUNELA, V. B., KULLMANN, A. & GEISLER, G. (1990) Nitrogen distribution and dry matter accumulation in oilseed rape (Brassica napus L.) as influenced by nitrogen nutrition. Journal of Agronomy and Crop Science, 164, 321-333.

OLIVER, I. W., MCLAUGHLIN, M. J. & MERRINGTON, G. (2005) Temporal trends of total and potentially available element concentrations in sewage biosolids: a comparison of biosolid surveys conducted 18 years apart. Science of the Total Environment, 337, 139-145.

OLIVER, I. W., MERRINGTON, G. & MCLAUGHLIN, M. J. (2004) Australian biosolids: Characterization and determination of available copper. Environmental Chemistry, 1, 116-124.

OTABBONG, E., ATTERWALL, S., PERSSON, J. & HAAK, E. (1996) Sewage sludge: Soil conditioner and nutrient source. I. Phosphorus availability and its uptake by ryegrass grown in a pot experiment.The Science of Composting. Part 2; . Blackie Academic and Professional:Glasgow, UK, .

OUEDRAOGO, E., MANDO, A. & ZOMBRE, N. P. (2001) Use of compost to improve soil properties and crop productivity under low input agricultural system in West Africa. Agriculture Ecosystems and Environment, 84 259–266.

OZER, H. (2003) Sowing date and nitrogen rate effects on growth, yield and yield components of two summer rapeseed cultivars. Europ. J. Agron., 19, 453-463.

OZORES-HAMPTON, M. (1998) Compost as an alternative weed control method. . Hort.Science 33 938–940.

OZORES-HAMPTON, M., HANLON, E., BRYAN, H. & SCHAFFER, B. (1997) Cadmium, copper, lead, nickel and zinc concentrations in tomato and squash grown in MSW compost-amended calcareous soil. Compost Science and Utilization 5 40-45.

PAILLAT, J.-M., ROBIN, P., HASSOUNA, M. & LETERME, P. (2005). Predicting ammonia and carbon dioxide emissions from carbon and nitrogen biodegradability during animal waste composting. Atmospheric Environment, 39**,** 6833-6842.

PAGE, A. L., LOGAN, T. J. & RYAN, J. A. (1987) Land application of sludge - food chain implications. Chelsea, USA: Lewis Publishers.

PARKINSON, R., FULLER, M., JURY, S. & GROENHOF, A. (1996) An evaluation of soil nutrient status following the application of (i) co-compostedMSWand sewage sludge and (ii) green waste to maize. In: M. De Bertoldi, P. Sequi, B. Lemmes, T. Papi (Eds.), The Science of Composting,. Blackie Academic & Professional, London.

PARKPAIN, P., SIRISUKHODOM, S., CARBONELL-BARRACHINA, A.A., 1998.Heavy metal and nutrient chemistry in sewage sludge amended Thai soils.J.Envir on. Sci. Health 33, 573–597.

PASTENE, A. J. (1981) Factors Affecting the Crop Availability of Phosphorus in Sewage Sludge Amended Soils. M.Sc. thesis,University of Wisconsin,Madison, WI.

PATRIQUIN, D. G., BLAIKIE, H., PATRIQUIN, M. J. & YANG, C. (1993) On farm measurement of pH, electrical conductivity and nitrate in soil extracts for monitoring coupling and decoupling of nutrient cycles. Biological Agric. Hortic., 9, 231-272.

PAUL, E. A. & CLARK, F. E. (1996) Soil Microbiology and Biochemistry, 2nd ed. Academic Press Inc., San Diego, CA, 188 pp.

PAYMENT, P. & FRANCO, E. (1993) Clostridium perfringens and somatic coliphages as indicators of the efficiency of drinking water treatment for viruses and protozoan cysts. Applied and Environmental Microbiology, 59, 2418-2424.

PAYMENT, P., PLANTE, R. & CEJKA, P. (2001) Removal of indicator bacteria, human enteric viruses, Giardia cysts, and Cryptosporidium oocysts at a large wastewater primary treatment facility. Canadian Journal of Microbiology, 47, 188-193.

PEARSON, G. A. (1960) Tolerance of crops to exchangeable sodium. USDA Information Bulletin 216. USDA Government Printing Office, Washington DC.

PEDERSEN, D. C., PINCINCE, A. B. & BATES, J. E. (1981) Reduction of bacteria, viruses and parasites during conventional sludge treatment processes. Water Pollution Control Federation 54th Annual Conference.

PENN, C. J. & SIMS, J. T. (2002) Phosphorus forms in biosolids-amended soils and losses in runoff: effects of wastewater treatment process. Journal of Environmental Quality, 31, 1349-1361.

PENNINGTON, T. H. (2001) Pathogens in agriculture and the environment. In: Pathogens in Agriculture and the Environment, Meeting organised by the SCI Agriculture and Environment Group. SCI, London.

PEPPER, I. L., JOSEPHSON, K. L., BAILEY, R. L., BURR, M. D. & GERBA, C. P. (1993) Survival of indicator organisms in Sonoran Desert soil amended with sewage sludge. J. Environ. Sci.Health, A28, 1287–1302.

PEPPER, I. L., ZERZGHI, H., BROOKS, J. P. & GERBA, C. P. (2008). Sustainability of Land Application of Class B Biosolids. *Journal of Environmental Quality,* 37**,** S-58-S-67.

PEREZ-MURCIA, M. D., MORAL, R., MORENO-CASELLES, J., PEREZ-ESPINOSA, A. & PAREDES, C. (2006) Use of composted sewage sludge in growth media for broccoli. Bioresource Technology, 97, 123-230.

PETERSON, A. E., SPETH, P. E., COREY, R. B., WRIGHT, T. H. & SCHLECT, P. L. (1994) Effect of twelve years of liquid digested sludge applicationson soil phosphorus levels. In C.E. Clapp et al. (ed.) Sewage sludge: Land utilization and the environment., SSSA,Madison, WI.

PEVERILL, K. I., SPARROW, L. A. & REUTER, D. J. (1999) Soil Analysis: An Interpretation Manual, Collingwood, Australia., CSIRO Publishing.

PEVERLY, J. H. & GATES, P. B. (1994) Utilization of municipal solid waste and sludge composts in crop production systems. In: Clapp CE, Larson WE, Dowdy RH, editors. Sewage Sludge: Land Utilisation and the Environment (SSSA Miscellaneous Publication). Madison 7 Soil Science Society of America.

PIERZYNSKI, G. M. (1994) Plant nutrient aspects of sewage sludge. In C.E. Clapp et al. (ed.) Sewage sludge: Land utilization and the environment. SSSA, Madison WI.

PIETRONAVE, S., FRACCHIA, L., RINALDI, M. & MARTINOTTI, M. G. (2004) Influence of biotic and abiotic factors on human pathogens in a finished compost. Water Research, 38, 1963-1970.

PIETRZAK, U. & MCPHAIL, D. C. (2004) Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. Geoderma, 122, 151-166.

PIKE, E.B. & CARRINGTON, E.G. (1986) Stabilization of sludge by conventional and novel processes – a healthy future. In The Agricultural Use of Sewage Sludge – Is there a Future? London: The Institute of Water Pollution Control.

PITERINA, V.A., BARTLETT, J. & PEMBROKE, J.T. (2010) Evaluation of the Removal of Indicator Bacteria from Domestic Sludge Processed by Autothermal Thermophilic Aerobic Digestion (ATAD). Int. J. Environ. Res. Public Health ,7, 3422-3441

PLACHY, P. (1995) Helminths eggs in processes of treatment municipal wastewaters. PhD Thesis, Parasitological Institute of the Slovak Academy of Sciences, Kosice, Slovak Republic.

POINT, P. (1994) The value of non-market natural assets as production factor. In : R. Pethig, ed., Valuing the Environment: Methodological and Measurement Issues. Kluwer, Netherlands.

POLGASE, P. J. & ROBINSON, M. B. (1996) Sustainable Use of Biosolids in Plantation Forests, 1st Int. Conf. - Contaminants and Soil Environ., Adelaide.

PONUGOTI, R.P., M.F. DEHAB, F.M. & R. SURAMPALLI, R. (1997) Effects of different biosolids treatment systems on pathogen and pathogen indicator reduction. Water Environment Research, 69(7), 1195.

POTTER, T., MARCROFT, S., WALTON, G. & PARKER, P. (1999) Climate and soils. In : Salisbury, P.A., Potter, T.D., McDonald, G and Green A.G. (Eds). Canola in Australia, the first thirty years.Organising committee of the 10th International Rapeseed Congress, p. 3-5.

POTTS, P. (2004) Soil and Environmental analysis: modern techniques, chapter 7- X-ray fluorescence analysis, Open University, Marcel Dekker.

POTTS, P. J. (1998) A perspective on the evolution of geoanalytical techniques for silicate rocks. Geostandards Newsletter, 22, 57-68.

POURCHER, A. M., FRANCOISE, P. B., FERRE, V., GOSINSKA, A., STAN, V. & MOGUEDET, G. (2007) Survival of faecal indicators and enteroviruses in soil after land-spreading of municipal sewage sludge Applied Soil Ecology, 35, 473–479.

POURCHER, A. M., MORAND, P., PICARD-BONNAUD, F., BILLAUDEL, S., MONPOEHO, S., FEDERIGHI, M., FERRE, V. & MOGUEDET, G. (2005) Decrease of enteric micro-organisms from rural sewage sludge during their composting in straw mixture. Journal of Applied Microbiology, 99, 528-539.

PRESCOTT, L. M., HARLEY, J. P. & KLEIN, D. A. (2002) *Microbiology, 5th edn, McGraw-Hill, New York.*

PRITCHARD, D. (2005) Phosphorus bioavailability in land applied biosolids in South Western Australia, Ph.D Dessertation. Curtin University of Technology, Murek Institute.

PRITCHARD, F. M., EAGLES, H. A., NORTON, R. M., SALISBURY, P. A. & NICOLAS, M. (2000) Environmental effects on seed composition of Victorian canola. Australian Journal of Experimental Agriculture, 40, 679–685.

QASIM, M., JAVED, N. & HIMAYATULLAH, S. M. (2001) Effect of sewage sludge on the growth of maize crop. J. biol.Sci., 1, 52-54.

QIAN, J., WANG, Z.-J., SHAN, X.-Q., TU, Q., WEN, B. & CHEN, B. (1996) Evaluation of plant availability of soil trace metals by chemical fractionation and multiple regression analysis. Environmental Pollution, 91, 309-315.

QUIRK, J. P. & POSNER, A. M. (1975) Trace element adsorption by soil minerals. 95-107.

QURESHI, S., RICHARDS, B. K., HAY, A. G., TSAI, C. C., MCBRIDE, M. B., BAVEYE, P. & STEENHUIS, T. S. (2003) Effect of Microbial Activity on Trace Element Release from Sewage Sludge. Environmental Science and Technology, 37, 3361-3366.

RAPPAPORT, B. D., MARTENS, D. C., RENEAU, R. B., JR. & SIMPSON, T. W. (1988) Metal availability in sludge-amended soils with elevated metal levels. Journal of Environmental Quality, 17, 42-47.

RASMUS, F. PETER, O., MARIA, G. ÅSTRÖM, MATS, A. (2009) Metal concentrations in oats (Avena sativa L.) grown on acid sulphate soils. Agriculture and Food science, 18, 45-56.

RAVEN, K. P. & LOEPPERT, R. H. (1997) Trace element composition of fertilizers and soil amendments. Journal of Environmental Quality, 26, 551-557.

RAYMENT, G. E. & HIGGINSON, F. R. (1992) Australian Laboratory Handbook of Soil and Water Chemical Methods, Australian Soil and Land Survey Handbook, Inkarta Press, Australia.

REDDY, K. R., KHALEEL, R. & OVERCASH, M. R. (1981) Behavior and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. J. Environ.Sci. Health A28, 1287-1302.

REDEL, Y. D., RUBIO, R., ROUANET, J. L. & BORIE, F. (2007) Phosphorus bioavailability affected by tillage and crop rotation on a Chilean volcanic derived Ultisol. Geoderma, 139, 388-396.

REID, H. (2002) Key guideline requirements for biosolids land application: The basis and practical implications, EPA Victoria 65th Annual Water Industry Engineers and Operators' Conference Kardinia Heights Centre-Geelong.

REUTER, D. J. & ROBINSON, J. B. (1997) Plant analysis: An Interpretation Manual, Collingwood, Australia, CSIRO Publishing.

RICHARDS, B. K., PEVERLY, J. H., STEENHUIS, T. S. & LIEBOWITZ, B. N. (1997) Effect of processing mode on trace elements in dewatered sludge products. Journal of Environmental Quality, 26, 782-788.

RICHARDS, B. K., STEENHUIS, T. S., PEVERLY, J. H. & MCBRIDE, M. B. (2000) Effect of sludge-processing mode, soil texture and soil pH on metal mobility in undisturbed soil columns under accelerated loading. Environmental Pollution (Oxford, United Kingdom), 109, 327-346.

RICHARDS, B.K., STEENHUIS, T.S. & MCBRIDE, M.B., (2001). Metal mobility from sludge applied to undisturbed soil columns. In: Doner, H., Carini, F., Selim, M., Mench, M., Chen, Z.S., Luo, Y. (Eds.), Sixth International Conference on the Biogeochemistry of Trace Elements.11 –15 July, Guelph. International Society for Trace Element Research, Vienna, Austria.pp. 163.

ROBERT THOMAS (2004) Practical Guide to ICP-MS Scientific Solutions, Gaithersburg, Maryland, U.S. A.

ROBERTSON, W. K., LUTRICK, M. C. & YUAN, T. L. (1982) Heavy applications of liquid-digested sludge on three ultisols: I. Effects on soil chemistry. Journal of Environmental Quality, 11, 278-782.

ROBINSON, J. (2001) A review of techniques to value environmental resources in coastal zones milestone report. CRC for Coastal Zone Estuary and Waterway Management, University of Queensland.

ROBINSON, M. B., POLGLASE, P. J. & WESTON, C. J. (2002) Loss of mass and nitrogen from biosolids applied to pine plantation. Australian Journal of Soil Science, 40, 1027-1039.

RODRIGUES, M., LOPEZ-REAL, J. & LEE, H. (1996) Use of composted societal organic wastes for sustainable crop production In: M. De Bertoldi, P. Sequi, B. Lemmes, T. Papi (Eds.), The Science of Composting. Blackie Academic & Professional, London.

RODRIGUEZ, M. B., MAGGI, L., ETCHEPAREBORDA, M., TABOADA, M. A. & LAVADO, R. S. (2003) Nitrogen availability for maize from a rolling pampa soil after addition of biosolids. Journal of Plant Nutrition, 26, 431-441.

ROE, N. E. (1998) Compost utilization for vegetable and fruit crops. HortScience 33, 934–937.

ROKA, F. M., MUCHOVEJ, R. M. & OBREZA, T. A. (2004) Assessing Economic Value of Biosolids. U.S. Department of Agriculture, Cooperative Extension Service,, University of Florida, IFAS, Florida A. & M. University Cooperative Extension Program, and Boards of County Commissioners Cooperating. Larry Arrington, Dean.
.
ROSS, A. D., LAWRIE, R. A., WHATMUFF, M. S., KENEALLY, J. P. & AWAD, A. S. (1991) Guidelines for the use of sewage sludge on agricultural land, New South Wales Agriculture.

RUDOLFS, W., FALK, L. L. & RAGOTZKIE, R. A. (1950) Literature review on the occurrence and survival of enteric, pathogenic, and related organisms in soil, water, sewage, and sludges, and on vegetation. II. Animal parasites. Sewage and Industrial Wastes, 22, 1417-1427.

RUSS, C. F. & YANKO, W. A. (1981) Factors affecting salmonellae repopulation in composted sludges. Applied and Environmental Microbiology, 41, 597-602.

RYDIN, E. & OTABBONG, E. (1997) Potential release of phosphorus from soil mixed with sewage sludge. Journal of Environmental Quality, 26, 529-534.

SADOVNIKOVA, L., OTABBONG, E., IAKIMENKO, O., NILSSON, I., PERSSON, J., ORLOV, D., (1996). Dynamic transformation of sewage sludge and farmyard manure components.2. Copper. lead and cadmium forms in incubated soils.Agric. Ecosyst. Environ.58, 127–132.

SA EPA (1997) South Australian biosolids guidelines for the safe handling, reuse or disposal of biosolids. South Australian Adelaide, SA., Department of Environment and Natural Resources.

SAHLSTROM, L. (2003) A review of survival of pathogenic bacteria in organic waste used in biogas plants. Bioresource Technology, 87, 161-166.

SAHLSTROM, L., ASPAN, A., BAGGE, E., DANIELSSON-THAM, M.-L. & ALBIHN, A. (2004) Bacterial pathogen incidences in sludge from Swedish sewage treatment plants. Water Research, 38, 1989-1994.

SALISBURY, P. & WRATTEN, N. (1999) Brassica napus breeding. In : Salisbury, P.A., Potter, T.D., McDonald, G and Green A.G. (Eds). Canola in Australia, the first thirty years. Organising committee of the 10th International Rapeseed Congress, p.29-35.

SALSALI, H., PARKER, W. J. & SATTAR, S. A. (2008) The effect of volatile fatty acids on the inactivation of Clostridium perfringens in anaerobic digestion. World Journal of Microbiology & Biotechnology, 24, 659-665.

SAMARAS, V. & TSADILAS, C. D. (1997) Distribution and availability of six heavy metals in a soil treated with sewage sludge. In Proc. Int. Conf. Biogeochemistry of Trace Elements. Berkeley, CA, 145.

SAMUEL, S. C., HANCOCK, P. & LEIGH, D. A. (1991) An investigation into Clostridium perfringens enterotoxin associated diarrhoea. *J Hosp Infect* 18**,** 219-230.

SANTOS, I. & BETTIOL, W. (2003) Effect of sewage sludge on the rot and seedling damping-off of bean plants caused by Sclerotium rolfsii. Crop Protection, 22, 1093–1097.

SARKAR, B. (2002) Heavy Metals in the Environment .The Hospital for Sick Children and University of Toronto, Toronto, Ontario, Canada.

SARKAR, D. & O'CONNOR, G. A. (2004) Plant and Soil Responses to Biosolids-Phosphorus in Two Florida Soils with High Phosphorus Content. Communications in Soil Science and Plant Analysis, 35, 1569-1589.

SATHIYA BAMA, K., SELVAKUMARI, G., SANTHI, R. & SINGARAM, P. (2003) Effect of humic acid on nutrient release pattern in an Alfisol (Typic Haplustalf), Madras Agric. J. 90 (10-12): 665-670

SCHAFER, P. L. & FARRELL, J. H. (2000) Turn up the heat. Water Environment & Technology, 12, 26-32.

SCHEUERMAN, P. R., FARRAH, S. R. & BITTON, G. (1991) Laboratory studies of virus survival during aerobic and anaerobic digestion of sewage sludge. Water Research, 25, 241-245.

SCHLOTZ, R. & UHLIG, S. (2002) Introduction to X-ray fluorescence analysis (XRF) - User's manual, Bruker advanced x-ray solutions, Karsruhe, West Germany.

SCOTT, M. J., VAIL, L. W., JAKSCH, J. A., ANDERSON, K. K., STÖCKLE, C. O. & EVANS, R. G. (1999) Early Warning of ENSO Events for Regional Agriculture. PNNL-SA-31245 . Presented at the NOAA Economics and Human Dimensions Investigators Meeting, Tucson, Arizona, April 26, 1999. Pacific Northwest National Laboratory, Richland, Washington.

SCOTT, R. K., OGUNREMI, E. A., IVINS, J. D. & MENDHAM, N. J. (1973) Effect of fertilizers and harvest date on growth and yield of oilseed rape sown in autumn and spring. Journal of Agricultural Science, 81, 287-293.

SEARLE, P. L. (1984) The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen. A review. Analyst (Cambridge, United Kingdom), 109, 549-568.

SEPAC (1995) Environmental quality standards for soils. State Environmental Protection Administration of China, GB15618-1995. Sewage Sludges and Sludge-Treated Soils. Adv. Soil Sci., 9, 143–176.

SHARPLEY, A. N. & BEEGLE, D. (1999) Managing phosphorus for agriculture and the environment. , Coop. Ext. Serv., Pennsylvania State Univ., University Park.

SHEPPARD, S. C. & BATES, T. E. (1980) Yield and chemical composition of rape in response to nitrogen, phosphorus and potassium. ***Can. J. Soil Sci.*** , 60**,** 153 -162.

SHOBER, A. L., STEHOUWER, R. C. & MACNEAL, K. E. (2003) On-farm assessment of biosolids effects on soil and crop tissue quality. J. Environ. Qual. FIELD Full Journal Title:Journal of Environmental Quality, 32, 1873-1880.

SHOBER, A. L., STEHOUWER, R. C. & MACNEAL, K. E. (2007) Chemical fractionation of trace elements in biosolid-amended soils and correlation with trace elements in crop tissue. Communications in Soil Science and Plant Analysis, 38, 1029-1046.

SHUMAN, L. M. (1975) Effect of soil properties on zinc adsorption by soils. Soil Science Society of America Proceedings, 39, 454-458.

SIDHU, J., GIBBS, R. A., HO, G. E. & UNKOVICH, I. (2001) The role of indigenous microorganisms in suppression of Salmonella regrowth in composted biosolids. Water Research, 35, 913-920.

SIDHU JATINDER, P. S. & TOZE SIMON, G. (2009) Human pathogens and their indicators in biosolids: a literature review. Environment international, 35, 187-201.

SINGH, C. P. & AMBERGER, A. (1990) Humic substances in straw compost with rock phosphate. Biological Wastes, 31**,** 165-174.

SIKORA, L. J., MILLNER, P. D. & BURGE, W. D. (1985) Chemical and Microbial Aspects of Sludge Composting and Land Application. Report to U.S. EPA Office of Research and

Development, . Cincinnati, Ohio, Interagency Agreement Number AD-12-F-2-534. U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland.

SIMS, J. T. (1990) Nitrogen mineralization and elemental availability in soils amended with cocomposted sewage sludge. Journal of Environmental Quality, 19, 669-675.

SIMS, J. T. & KLINE, J. S. (1991) Chemical fractionation and plant uptake of heavy metals in soils amended with co-composted sewage sludge. Journal of Environmental Quality, 20, 387-395.

SINGH, R. P. & AGRAWAL, M. (2007) Potential benefits and risks of land application of sewage sludge. Waste Management (Amsterdam, Netherlands), 28, 347-358.

SINGH, S. D. & YUSUF, M. (1979) The effect of water and nitrogen and row spacing on yield and oil content of brown sarson. Can.J.Plant Sci., 59, 437-444.

SIVASITHAMPARAM, K., BARBETTI, M. J. & LI, H. (2005) Recurring challenges from a necrotrophic fungal plant pathogen: a case study with Leptosphaeria maculans (causal agent of blackleg disease in Brassicas) in Western Australia. Ann. Bot. , 96 363-377.

SKANAVIS, C. & YANKO, W. A. (1994) Evaluation of composted sewage sludge based soil amendments for potential risks of salmonellosis. J Environ Health, 56, 19–23.

SKINNER, P. W., MATTHEWS, M. A. & CARLSON, R. M. (1987) Phosphorus requirements of wine grapes: extractable phosphate of leaves indicates phosphorus status. J. Am. Soc. Hortic. Sci. FIELD Full Journal Title:Journal of the American Society for Horticultural Science, 112, 449-454.

SMIDT, E., MEISSL, K., SCHMUTZER, M. & HINTERSTOISSER, B. (2008) Co-composting of lignin to build up humic substances--Strategies in waste management to improve compost quality. Industrial Crops and Products, 27**,** 196-201.

SMITH, K.A. & CONEN, F. (2004) Impacts of land management on fluxes of trace greenhouse gases. *Soil Use* Manage. 20, 255–263.

SMITH, S.R. & HALL, J.E. (1991) Some economic and quality aspects of composting sewage sludge. In: Colin F, Newman PJ, Poulanne YJ, editors. Recent developments in sewage sludge processing. Barking: Elsevier Applied Science Publishers Ltd; p. 46–62.

SMITH, C. J., WRIGHT, G. C. & WOODROOFE, M. R. (1988) The effect of irrigation and nitrogen fertilizer on rapeseed (Brassica napus) production in south-eastern Australia. II. Nitrogen accumulation and oil yield. Irrigation Science, 9, 15-25.

SMITH, J. E., YOUNG, K. W. & DEAN, R. B. (1975) Biological oxidation and desinfection of sludge. Water Research, 9, 17.

SMITH, S.R. (1994a) Effect of soil pH on availability to crops of metals in sewage sludge-treated soils. I. Nickel, copper and zinc uptake and toxicity to ryegrass. Environ Pollut 85:321–7.

SMITH, S.R. (1994b) Effect of soil pH on availability to crops of metals in sewage sludge-treated soils. II. Cadmium uptake by crops and implications for human dietary intake.
Environ Pollut 86:5–13.

SMITH, S. R. (1996) Agricultural Recycling of Sewage Sludge and the Environment. CAB International, Wallingford, UK.

SMITH, S. R., TIBBETT, M. & EVANS, T. D. (1992) Nitrate accumulation potential of sewage sludge applied to soil. Nitrate Farm. Syst., Aspects Appl. Biol. 30, 157-161.

SMITH, M. T. E. & TIBBETT, M. (2004) Nitrogen dynamics under Lolium perenne after a single application of three different sewage sludge types from the same treatment stream. Bioresource Technology, 91, 233-241.

SMITH, S.R. (2009) A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. Environment international, 35, 142-156.

SMOLDERS, E., BUEKERS, J., OLIVER, I. & MCLAUGHLIN, M. J. (2004) Soil properties affecting toxicity of zinc to soil microbial properties in laboratory-spiked and field-contaminated soils. Environmental Toxicology and Chemistry, 23, 2633-2640.

SNOWDON, J. A., CLIVER, D. O. & CONVERSE, J. C. (1989) Land disposal of mixed human and animal wastes: a review. Waste Management & Research, 7, 121-134.

SOLAIMAN, Z., MARSCHNER, P., WANG, D. & RENGEL, Z. (2007) Growth, P uptake and rhizosphere properties of wheat and canola genotypes in an alkaline soil with low P availability. Biology and Fertility of Soils, 44, 143-153.

SOLTANPOUR, P. N. (1991) Determination of nutrient availability and elemental toxicity by AB-DTPA soil test and ICPS. Advances in Soil Science, 16, 165-190.

SOMMERS, L. E., PAGE, A. L., LOGAN, T. J. & RYAN, J. A. (1991) Optimum use of sewage sludge on agricultural land. Western Regional Res. Publ.W-124, Colorado Agric.Exp. Stn, Fort Collins

SOMMERS, L. E. (1977) Chemical composition of sewage sludges and analysis of their potential use as fertilizers. *J. Environ. Qual.,* 6**,** 225-232.

SOON, Y. K. & BATES, T. E. (1982) Extractability and solubility of phosphate in soils amended with chemically treated sewage sludges. Soil Science, 134, 89-96.

SOON, Y. K., BATES, T. E. & MOYER, J. R. (1978) Land application of chemically treated sewage sludge: II. Effects on plant and soil phosphorus, potassium, calcium, and magnesium and soil pH. Journal of Environmental Quality, 7, 269-273.

SORBER, C. A. & MOORE, B. E. (1986) Survival and transport of pathogens in sludge-amended soil . In: Proceedings of the National Conference on Municipal Treatment Plant Sludge Management. Orlando, Florida, information Transfer, Inc., Rockville, Maryland.

SORBER, C. A. & MOORE, B. E. (1987) Survival and transport of pathogens in sludge-amended soil: a critical literature review. (EPA/600/S2-87/028). US EPA. Cincinnati, Ohio.

SPARKS, D. L. (1996) Methods of Soil Analysis. Part 3. Chemical Methods ,Soil Science Society of America Book Series, Chap.30 pp 835.

SPEIR, T. W., KETTLES, H. A., PERCIVAL, H. J. & PARSHOTAM, A. (1999) Is soil acidification the cause of biochemical responses when soils are amended with heavy metal salts? Soil Biology & Biochemistry, 31, 1953-1961.

SPOSITO, G., LUND, L. J. & CHANG, A. C. (1982) Trace metal chemistry in arid-zone field soils amended with sewage sludge: I. Fractionation of nickel, copper, zinc, cadmium and lead in solid phases. Soil Science Society of America Journal, 46, 260-264.

STADEN, J. V., KULKARNI, M. G. & SPARG, S. G. (2005) Influence of temperature and watering frequencies on seed germination and seedling growth of Ornithogalum longibracteatum and Tulbaghia violacea Scientia Horticulturae, 107, 103–109.

STAMATIADIS, S., WERNER, M. & BUCHANAN, M. (1999) Field assessment of soil quality as affected by Compost and fertilizer application in a broccoli field (San Benito County, California). Applied Soil Ecology, 12, 217- 225.

STEVENS, D. P., MCLAUGHLIN, M. J. & HEINRICH, T. (2003) Determining toxicity of lead and zinc runoff in soils: Salinity effects on metal partitioning and on phototoxicity. Environmental Toxicology and Chemistry, 22, 3017-3024.

STEVENSON, F. C. & VAN KESSEL, C. (1996) A landscape-scale assessment of the nitrogen and non-nitrogen rotation benefits of pea. Soil Science Society of America Journal, 60, 1797-1805.

STEWART, D. P. C., CAMERON, K. C. & CORNFORT, I. S. (1998) Effects of spent mushroom substrate on soil chemical conditions and plant growth in an intensive horticultural system: a comparison with inorganic fertiliser. Australian Journal of Soil Research, 36 185–198.

STOKES, J., CODY, J., SURAPANENI, A., WRIGLEY, R. & NORNG, S. (5-9 December 2004) Soil and Crop Responses (Year 1) from Biosolid Trial at Dookie, Victoria. Proceedings of the International Soil Science Conference Sydney, Australia.

STOSKOPF, N. C. (1985) Cereal grain crops, Reston Publishing Company, Reston, Virginia.

STRAUB, T. M., PEPPER, I. L. & GERBA, C. P. (1992) Persistence of viruses in desert soils amended with anaerobically digested sewage sludge. Appl Environ Microbiol, 58, 636–641.

STRAUB, T. M., PEPPER, I. L. & GERBA, C. P. (1993) Hazards from pathogenic microorganisms in land-disposed sewage sludge. Reviews of environmental contamination and toxicology, 132, 55-91.

STRAUCH, D. (1987) Hygiene of animal waste management. Animal Production and Environmental Health World Animal Science, vol. B6.Elsevier Science Publishers B.V, New York.

STREET, J. J., SABEY, B. R. & LINDSAY, W. L. (1978) Influence of pH, phosphorus, cadmium, sewage sludge, and incubation time on the solubility and plant uptake of cadmium. Journal of Environmental Quality, 7, 286-290.

SU, D. C. & WONG, J. W. C. (2004) Chemical speciation and phytoavailability of Zn, Cu, Ni and Cd in soil amended with fly ash-stabilized sewage sludge. Environment international, 29, 895-900.

SU, J., WANG, H., KIMBERLEY, M. O., BEECROFT, K., MAGESAN, G. N. & HU, C. (2007) Fractionation and mobility of phosphorus in a sandy forest soil amended with biosolids. Environmental Science and Pollution Research, 14**,** 529-535.

SUI, Y., THOMPSON, M. L. & SHANG, C. (1999) Fractionation of phosphorus in a Mollisol amended with biosolids. Soil Science Society of America Journal, 63, 1174-1180.

SUN, Y. H., LUO, Y. M., WU, L. H., LI, Z. G., SONG, J. & CHRISTIE, P. (2006) Survival of faecal coliforms and hygiene risks in soils treated with municipal sewage sludges. Environmental geochemistry and health, 28, 97-101.

SURAMPALLI, R. Y., BANERJI, S. K. & CHEN, J. C. (1993). Microbiological Stabilization of Sludge by Aerobic Digestion and Storage. Journal of Environmental Engineering, 119**,** 493-505.

TANNER, B. D., BROOKS, J. P., GERBA, C. P., HAAS, C. N., JOSEPHSON, K. L. & PEPPER, I. L. 2008. Estimated Occupational Risk from Bioaerosols Generated during Land Application of Class B Biosolids. *Journal of Environmental Quality,* 37**,** 2311-2321.

TARAFDAR, J. C. & CLAASSEN, N. (1988) Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. Biology and Fertility of Soils, 5, 308-312.

TARRASON, D., OJEDA, G., ORTIZ, O. & ALCANIZ, J. M. (2007) Differences on nitrogen availability in a soil amended with fresh, composted and thermally-dried sewage sludge. Bioresource Technology, 99, 252-259.

TAYLOR, J. M., EPSTEIN, E., BURGE, W. D., CHANEY, R. L., MENZIES, J. D. & SIKORA, L. J. (1978) Chemical and biological phenomena observed with sewage sludges in simulated soil trenches. Journal of Environmental Quality, 7, 477-482.

TECHNICON INSTRUMENT CORPORATION (1971) Nitrate and Nitrite in Water. Industrial method No. 32–69 W. Technicon Instrument Corporation, Tarrytown, New York, NY.

THOMAS, P. (1984) Canola growers manual, Lacombe, AB, Canola Council of Canada.

TIESSEN, H., STEWART, J. W. B. & COLE, C. V. (1984) Pathways of phosphorus transformations in soils of differing pedogenesis. Soil Science Society of America Journal, 48, 853-858.

TRAINA, S. J., SPOSITO, G., HESTERBERG, D. & KAFKAFI, U. (1986) Effects of pH and organic acids on orthophosphate solubility in an acidic, montmorillonitic soil. Soil Science Society of America Journal, 50, 45-52.

TSADILAS, C. D., MATSI, T., BARBAYIANNIS, N. & DIMOYIANNIS, D. (1995) Influence of sewage sludge application on soil properties and on the distribution and availability of heavy metal fractions. Communications in Soil Science and Plant Analysis, 26, 2603-2619.

TSADILAS, C. D., MITSIOS, I. K. & GOLIA, E. (2005) Influence of biosolids application on some soil physical properties. Communications in Soil Science and Plant Analysis, 36, 709-716.

TSAKOU, A., ROULIA, M. & CHRISTODOULAKIS, N. S. (2001) Growth of cotton plants (Gossypium hirsutum) as affected by water and sludge from a sewage treatment plant. I. Plant phenology and development. Bulletin of Environmental Contamination and Toxicology, 66, 735-742.

TSAKOU, A., ROULIA, M. & CHRISTODOULAKIS, N. S. (2001) Growth of cotton plants (Gossypium hirsutum) as affected by water and sludge from a sewage treatment plant: II. Seed and fiber yield and heavy metal accumulation. Bulletin of Environmental Contamination and Toxicology, 66, 743-747.

TSAKOU, A., ROULIA, M. & CHRISTODOULAKIS, N. S. (2002) Growth of flax plants (Linum usitatissimum) as affected by water and sludge from a sewage treatment plant. Bulletin of Environmental Contamination and Toxicology, 68, 56-63.

TUOHEY, C. L. & ROBSON, A. D. (1980) The effect of cropping after medic and non-medic pastures on total soil nitrogen, and on the grain yield and nitrogen content of wheat. Australian Journal of Experimental Agriculture and Animal Husbandry 20, 220-228.

ULRICH, B. (1987) Stability, elasticity and resilience of terrestrial ecosystems with respect to matter balance. In: Schulze, E.D., Zwolfer, H (Eds.), Potentials and Limitations of Ecosystem Analysis. Springer, New York.

FAO (United Nations-Food and Agriculture Organization) (1998) Guidelines for Quality Management in Soil and Plant Laboratories. [Online] Available at: http:// www.fao.org/doc re p/
W7295E/ w7295e00.htm (accessed on March 2007).

US EPA (1980) Sewage sludge: Factors affecting the uptake of cadmium by food–chain crops grown on sludge–amended soils. W–124 SEA–CR Technical Res. Committee, U.S. Environmental Protection Agency Report No. SW- 882, Washington, D.C.

US EPA (1984) Definition and Procedure for the Determination of the Method Detection Limit. 40 CFR136.2 Appendix B. [Online] Available at: http:/ ca.water.usgs.gov/pnsp/rep/interpret/def. html (accessed on March 2007).

U.S. EPA (1987) Characterisation of municipal waste combustor ashes and leachates from municipal solid waste landfills and co-disposal sites, I-VII, 530-SW-87-028A-E, Washington DC, U.S.A.
US EPA (1990) Quality Assurance/Quality Control Guidance for Removal Activities: Sampling QA/QC Plan and Data Validation Procedures. Interim Final, EPA/ 540/G-90/004.

US EPA (1992) Technical Support Document for Reduction of Pathogens and Vector Attraction in Sewage Sludge, U.S. Environmental Protection Agency, . EPA R-93-004, Washington, D.C. Wiley, J.S., Pathogen survival in composting municipal wastes, J. Water Pollut. Control Fed. 34: 80–90.

US EPA (1993). Standards for the use or disposal of sewage sludge. Final rule. 40 CFR Parts 257, 403, 503. Fed. Regist. 58:9248–9415.

US EPA (1994) Land Application of Sewage Sludge – A Guide for Land Appliers on the Requirements of the Federal Standards for the Use or Disposal of Sewage Sludge. (40 CFR Part 503. EPA/ 831-B-93–002b), Washington, DC: Office of Enforcement and Compliance Assurance.

US EPA (1999) Biosolids Generation, Use and Disposal in the United States. (EPA530-R-99–009), Washington, DC: Office of Solid Waste and Emergency Response.

US EPA (2000) Biosolids technology fact sheet: Land application of biosolids. The U.S. Environmental Protection Agency, EPA 832-F-00-064, Office of Water, Washington, D.C.

U.S. EPA (2002) Biosolids: Frequently Asked Questions ,Accessed 27 September, 2008, <http://www.epa.gov/owm/mtb/biosolids/genqa.htm>.

VAN DER ZAAL, B. J., NEUTEBOOM, L. W., PINAS, J. E., CHARDONNENS, A. N., SCHAT, H., VERKLEIJ, J. A. & HOOYKAAS, P. J. (1999) Over expression of a novel Arabidopsis gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation. Plant physiology, 119, 1047-1055.

VAN VUUREN, P. J. J. & LUBBE, A. C. (2000) The influence of temperature on the germination of seed of a commercial Verbena hybrid. Proc. XXV IHC - Part 5. Acta Hort., 515, 185–192.

VAZQUEZ, C., BARBARO, N. & LOPEZ, S. (2003) XRF analysis of micronutrients in endive grown on soils with sewage sludge. X-Ray Spectrometry, 32, 57-59.

Virtual Canola Crop Website: http://www.australianoilseeds.com/agronomy centre/the virtual canola site Australian oil Seeds Federation, Industry Facts and Figures, 2009 accessed in June 2009.

WA DEP (2002) Western Australian guidelines for direct application of biosolids and biosolids products. Perth, WA.

WALTER, I., CUEVAS, G., GARCIA, S. & MARTINEZ, F. (2000) Biosolids effects on soil and native plant production in a degraded semiarid ecosystem in central Spain. Waste Management & Research, 18, 259-263.

WALTER, I., MARTINEZ, F., ALONSO, L. & GABRIELA CUEVAS, J. D. G. (2002) Extractable soil heavy metals following the cessation of biosolids application to agricultural soil. Environmental Pollution (Oxford, United Kingdom), 117, 315-321.

WANG, X., WANG, Z. & LI, S. (1995) The effect of humic acids on the availability of phosphorus fertilizers in alkaline soils. Soil Use and Management, 11**,** 99-102.

WANG, H. & STUANES, A. O. (2003) Heavy Metal Pollution in Air-Water-Soil-Plant System of Zhuzhou City, Hunan Province, China. Water, Air, and Soil Pollution, 147, 79-107.

WANG, Q-R., LI, Y-C. & KLASSEN, W. (2003) Effects of soil amendments at a heavy loading rate associated with cover crops as green manures on the leaching of nutrients and heavy metals from a calcareous soil. Journal of Environmental Science and Health, Part B Pesticides, Food Contaminants, and Agricultural Wastes, B38, 865-881.

WARD, R. L. & ASHLEY, C. S. (1976) Inactivation of poliovirus in digested sludge. Applied and Environmental Microbiology, 31, 921-930.

WARD, R. L., YEAGER, J. G. & ASHLEY, C. S. (1981) Response of bacteria in wastewater sludge to moisture loss by evaporation and effect of moisture content on bacterial inactivation by ionizing radiation. Applied and Environmental Microbiology, 41, 1123-1127.

WARMAN, P. R. & TERMEER, W. C. (2005) Evaluation of sewage sludge, septic waste and sludge compost applications to corn and forage: Ca, Mg, S, Fe, Mn, Cu, Zn and B content of crops and soils. Bioresource Technology, 96, 1029-1038.

WARNE, M. (2007) Soil quality guidelines for Cu and Zn for soils receiving biosolids. National Biosolids Research Program Biosolids specialty III conference, Melbourne.

WARNE, M., MCLAUGHLIN, M., DIANE, H., BELL, M., BROOS, K., WHATMUFF, M., BARRY, G., NASH, D., PRITCHARD, D., STEVENS, D., PU, G. & BUTLER, C. (2007) Draft position paper, Recommendations of the Australian National Biosolids Research Program on Biosolids Guidelines. Biosolids Specialty III Conference.

WATANABE, H., KITAMURA, T., OCHI, S. & OZAKI, M. (1997) Inactivation of pathogenic bacteria under mesophilic and thermophilic conditions. Water Science and Technology, 36, 25-32.

WATKINS, J. & SLEATH, K. P. (1981) Isolation and enumeration of Listeria monocytogenes from Sewage, Sewage Sludge and River Water. The Journal of applied bacteriology, 50, 1-9.

WEBBER, L. R. & HILLIARD, B. C. (1974) Agricultural use of sludge. Conference Proceedings - Research Program for the Abatement of Municipal Pollution under Provisions of the Canada-Ontario Agreement on Great Lakes Water Quality, 2, 120-142.

WEBER, J., KARCZEWSKA, A., DROZD, J., LICZNAR, M., LICZNAR, S., JAMROZ, E. & KOCOWICZ, A. (2007) Agricultural and ecological aspects of a sandy soil as affected by the application of municipal solid waste composts. Soil Biology & Biochemistry, 39, 1294-1302.

WEGGLER-BEATON, K., GRAHAM, R. D. & MCLAUGHLIN, M. J. (2003) The influence of low rates of air-dried biosolids on yield and phosphorus and zinc nutrition of wheat (Triticum durum) and barley (Hordeum vulgare). Australian Journal of Soil Research, 41, 293-308.

WEI, Y. & LIU, Y. (2005) Effects of sewage sludge compost application on crops and cropland in a 3-year field study. Chemosphere, 59, 1257-1265.

WEIGHTMAN, R. M., HEYWOOD, C., WADE, A. & SOUTH, J. B. (2004) Relationship between grain (1 -> 3, 1-> 4)-beta -D-glucan concentration and the response of winter-sown oats to contrasting forms of applied nitrogen. Journal of Cereal Science, 40, 81-86.

WELCH, R. M. (1981) The biological significance of nickel. Journal of Plant Nutrition, 3, 345-356.
Welch, R.M. (1995) Micronutrient nutrition of plants. Critical Reviews of Plant Sciences 14, 49–82.

WELCH W. R. (1995) The Oat Crop: Production and Utilization, London, Published by Chapman & Hall.

WEN, G., BATES, T.E., VORONEY, R.P., YAMAMOTO, T., CHIKUSHI, J. & CURTIN, D. (2002a) A yield control approach to assess phytoavailability of Zn and Cu in irradiated, composted sewage sludges and composted manure in field experiments: I. Zinc. Plant Soil 246:231–40.

WESTERN WATER (2003a) Western Water Biosolids Management Plan.

WESTERN WATER (2003b) Melton Wastewater Purification Plant, (Surbiton Park) WesternWater.
WESTERN WATER (2008) Environment report.

WILLIAM, C. H. (1967) Some factors affecting the mineralization of organic sulphur in soils *Plant and Soil,* 26**,** 205 -223.

WILLIAMS, D. E., VLAMIS, J., PUKITE, A. H. & COREY, J. E. (1987) Metal movement in sludge-amended soils: a nine-year study. Soil Science, 143, 124-131.

WILSON, P., COOKE, M., CAWLEY, J., GILES, L. & WEST, M. (1995) Comparison of the determination of copper, nickel and zinc in contaminated soils by x-ray fluorescence spectrometry and inductively coupled plasma spectrometry. X-Ray Spectrometry, 24, 103-108.

WITHERS, P. J. A., CLAY, S. D. & BREEZE, V. G. (2001) Phosphorus transfer in runoff following application of fertilizer, manure, and sewage sludge. Journal of Environmental Quality, 30, 180-188.

WRIGHT, G. C., SMITH, C. G. & WOODROOFE, M. R. (1988) The effect of irrigation and nitrogen fertilizer on rapeseed (Brassica napus) production in south eastern Australia. I. Growth and seed yield. Irrig.Sci, 9, 1-13.

XIONG, X., YAN-XIA, L., MING, Y., FENG-SONG, Z. & WEI, L. (2010) Increase in complexation ability of humic acids with the addition of ligneous bulking agents during sewage sludge composting. Bioresource Technology, 101**,** 9650-9653.

YASARI, E., PATWARDHAN, A.M., GHOLE, V.S., OMID, G.C. & AHMAD, A. (2008). Relationship of growth parameters and nutrients uptake with canola (*Brassica napus* L.) yield and yield contribution at different nutrients availability. Pak. J. Biol. Sci., 11: 845-853.

YUAN, G. & LAVKULICH, L. M. (1997) Sorption behavior of copper, zinc, and cadmium in response to simulatechanges in soil properties. Communications in Soil science and Plant analysis, 28**,** 571-587.

ZAIER, H., GHNAYA, T., BEN REJEB, K., LAKHDAR, A., REJEB, S. & JEMAL, F. (2010). Effects of EDTA on phytoextraction of heavy metals (Zn, Mn and Pb) from sludge-amended soil with Brassica napus. Bioresource Technology, 101**,** 3978-3983.

ZALESKI, K. J., JOSEPHSON, K. L., GERBA, C. P. & PEPPER, I. L. (2005) Potential regrowth and recolonization of salmonellae and indicators in biosolids and biosolid-amended soil. Applied and Environmental Microbiology, 71, 3701-3708.

ZERZGHI, H., GERBA, C. P., BROOKS, J. P. & PEPPER, I. L. 2010. Long-Term Effects of Land Application of Class B Biosolids on the Soil Microbial Populations, Pathogens, and Activity. *Journal of Environmental Quality,* 39**,** 402-408.

ZAMBELLO, F. R. & ENZWEILER, J. (2002) Multi-element analysis of soils and sediments by wavelength-dispersive x-ray fluorescence spectrometry. Journal of Soils and Sediments, 2, 29-36.

ZHANG, M., HEANEY, D., HENRIQUEZ, B., SOLBERG, E. & BITTNER, E. (2006) A four-year study on influence of biosolids/MSW cocompost application in less productive soils in Alberta: nutrient dynamics. Compost Science & Utilization, 14, 68-80.

ZHANG, M., HEANEY, D., SOLBERG, E. & HERIQUEZ, B. (2000) The effect of MSW compost on metal uptake and yield of wheat, barley and canola in less productive farming soils of Alberta. Compost Sci. Utilization, 8, 224–235.

ZHANG, Q., TOM, W. S. & ANDREW, P. V. C. (2004) Leaching of nutrients from soil cores treated with a single large dose of digested sewage sludge. Biol Fertil Soils, 40, 284–289.

ZHAO, F. J., DUNHAM, S. J. & MCGRATH, S. P. (1997) Lessons to be learned about soil–plant metal transfers from the 50-year sewage sludge experiment at Woburn, UK. In Extended Abstracts of the 4th International Conference on the Biogeochemistry of Trace Elements. University of California, Berkeley, USA.

ZENNARO, M., CRISTOFORI, F., FORMIGONI, D., FRIGNANI, F &, PAVONI, B. (2005) Heavy metal contamination in compost. A possible solution. Ann Chim-Rome 95:247–56.

ZHELJAZKOV, V. D. & WARMAN, P. R. (2004) Phytoavailability and fractionation of copper, manganese, and zinc in soil following application of two composts to four crops. Environmental Pollution (Amsterdam, Netherlands), 131, 187-195.

ZWARICH, M. A. & MILLS, J. G. (1982) Heavy metal accumulation by some vegetable crops grown on sewage sludge-amended soils. Canadian Journal of Soil Science, 62, 243-247.

ZUFIAURRE, R., OLIVAR, A., CHAMORRO, P., NER´IN, C., CALLIZO, A., 1998.Speciation of metals in sewage sludge for agricultural uses.Analyst 123, 255–259.

APPENDICES

APPENDIX A

Calculations of biosolids loading rates based on nitrogen limited biosolids application rates (NLBAR)

Based on the data presented in chapter 4 Table 4.1, for concentrations of total-N, NH_4-N and NO_3-N in dewatered and composted biosolids, the nitrogen limited biosolids application rates (NLBAR) and their equivalent biosolids loading rates (t/ha) were calculated for both biosolids and crop types (NSW EPA, 1997). Available N requirement of 100 and 110 kgN/ha/yr for canola and oats, respectively were taken into account while calculating the NLBAR. Since the biosolids were stockpiled for over two years period, it was also assumed that some of the NH_4-N fractions may have been lost through ammonia volatilization, and hence the concentrations of the NH_4-N measured in the biosolids were taken as it was while calculating the available nitrogen in the biosolids (ABN).

Calculation of NLBAR for dewatered biosolids (2006)

$$NLBAR(t/ha) = \frac{CNR(kg/ha)}{ABN(kg/t)}$$

ABN (kg/t) = $(NH_4 - N) + (Oxidizable - N) + (TN - Inorganic - N) \times MR$

ABN (kg/t) = $[NH_4 - N + NO_3 - N] + [TN - (NH_4 - N + NO_3 - N)] \times 15\%$

Where CNR = crop nitrogen requirement, ABN = available biosolids nitrogen and MR= mineralization rates respectively. Hence, assuming 15 % mineralization rates of organic-N during the first year, the values of TN, NH_4-N and NO_3-N (mg/kg) were inserted in the above equation and gives:

ABN (kgN/t) = $[3735 + 830] + [42100 - (3735 + 830)] \times 15\%$

ABN (kgN/t) = $[4565] + [5630.25] \times 15\%$
ABN (kgN/t) = $[10195 mg / kg]$
ABN (kgN/t) = 10 kg N/ t of dewatered biosolids

$$NLBAR(t / ha) = \frac{CNR(kg / ha)}{ABN(kg / t)}$$

$NLBAR = \frac{100 kgN / ha}{10 kgN / t)}$ = 10 t/ha dewatered biosolids for canola crop

$NLBAR = \frac{110 kgN / ha}{10 kgN / t)}$ = 11 t/ha dewatered biosolids for oats crop

Calculation of NLBAR for composted biosolids (2006)

The same procedure was followed in the calculation of 1 NLBAR for canola and oat crops using composted biosolids, however 10 % organic–N mineralization rates during the first year of the experiment was assumed in the computation.

ABN (kgN/t) = $[NH_4 - N + NO_3 - N] + [TN - (NH_4 - N + NO_3 - N)] \times 10\%$

ABN (kgN/t) = $[2113 + 1864] + [14300 - (2113 + 1864)] \times 10\%$

ABN (kgN/t) = $[3977] + [10{,}323] \times 10\%$

ABN (kgN/t) = 5009.3 mg/kg

ABN (kgN/t) = 5 kg N/t of composted biosolids

$$NLBAR(t / ha) = \frac{CNR(kg / ha)}{ABN(kg / t)}$$

$NLBAR(t / ha) = \frac{100(kg / ha)}{5(kg / t)} = 20 t / ha$ composted biosolids for canola

$NLBAR(t / ha) = \frac{110(kg / ha)}{5(kg / t)} = 22 t / ha$ composted biosolids for oat

Biosolids were incorporated into 10 cm soil depth based on multiples of the calculated NLBAR for canola and oats crops.

In the 2007 experiment, dewatered and composted biosolids were applied at the same rates as in 2006, however 6 and 5 % organic-N mineralization rates of dewatered and composted biosolids applied in 2006 was assumed to continue in 2007. The amount of this expected organic-N to mineralize in dewatered and composted biosolids was: 6 % × Organic-N in dewatered biosolids;

6 % × 37535 mgN/kg = 2252 mgN/kg ~ 2.25kgN/t of dewatered biosolids, whereas for composted biosolids 5 % ×Organic-N in composted biosolids.

5% × 10323 mgN/kg = 516.15 mgN/kg ~ 0.52 kgN/t of composted biosolids.

Thus, the expected organic-N mineralization in 2007 from 2006 applied biosolids (6 and 5 % of the organic-N in dewatered biosolids and composted biosolids, respectively was added in the 2007 repeated biosolids application.

APPENDIX B

Comparisons between WD-XRF and ICP-MS determined total heavy metal residuals in biosolids amended soil

Introduction

This appendix compares the concentrations of total heavy metals in biosolids amended soils determined using WD-XRF and ICP-MS. The “total” heavy metals determined by ICP-MS were extracted using a solution of concentrated (70 %) nitric acid and (30 %) hydrogen peroxide (HNO_3/H_2O_2), whereas dry biosolids amended soil samples were analysed using WD-XRF.

The two analytical instruments were compared to determine the reproducibility (precision) and accuracy of the soil analytical data and quantify the relationship between the two instruments. This evaluation would help to choose an instrument that could be suitable for routine laboratory analysis of biosolids amended soil samples.

After harvesting canola and oats crops in the 2006 field experiment, thirty soil cores per plot at 10 cm soil depth were sampled and made into one composite sample, hence a total of 72 samples were taken from the 72 dewatered and composted biosolids amended canola and oats plots. Samples were analysed based on the analytical procedures mentioned in chapter 3 under section 3.7 and 3.8 for ICP-MS and XRF respectively.

B.1. ICP-MS analysis

ICP-MS is a powerful technique for the simultaneous determination of metals in aqueous solution (Hall, 1993). It uses plasma to ionize sample components which its quadrupole discriminates according to mass.

Sample introduction is accomplished by direct aspiration of small liquid volumes. The analysis time is rapid allowing replicate analysis, external and internal standardization and quality control procedures in line with the U.S. Environmental Protection Agency’s guidelines (USEPA, 1990).

The most frequent types of environmental samples being analysed using ICP-MS today for trace element determinations include drinking waters, ground waters, wastewaters, river waters, estuarine waters, seawaters, solid waste, soils, sludges, sediments, and airborne particulates (Robert, 2004).

B.1.1. Analytical quality assurance

To validate the analytical data for HNO3 /H2O2 extractable pseudo total heavy metals determined using ICP-MS, certified reference material CRM 031-040 (sewage sludge) was analysed in duplicate for every 18 batch of biosolids amended soil samples. Appendix D Table D-5 shows details of the recoveries of each of the heavy metals, whereas appendix Table B-1 summarizes the recoveries of Cu, Zn, Mn, Fe, Ni and Pb along with their certified values.

The minimum recoveries (65 and 82 %) were observed for Ni and Pb respectively, whereas the maximum recovery for Zn was 119 %; similarly Mn and Fe had relatively comparable maximum recoveries of 96 and 94 % respectively. Generally, a 100 % recovery for all the heavy metals was not expected because the certified reference material (CRM030-041) was supposed to be digested with HCl, HNO3, HF and HClO4 acid mixtures, but in this case, the reference material was digested using HNO_3/H_2O_2 solution.

Table B-1 Recoveries of 'total' metals in CRM031-040 (Sewage sludge standard reference material) extracted using HNO_3 /H_2O_2 and analysed by ICP-MS for data validation purposes (expressed in mg/kg).

Elements	Measured	Certified	Recovery (%)
Cu	727 ± 23	805 ± 91.1	88-90
Zn	1300 ± 100	1060 ± 88.6	88-119
Mn	190 ± 60	199 ± 24.6	89-96
Fe	9300 ± 100	9810 ± 824	88-94
Ni	17.4 ± 0.1	19.60 ± 3.34	65-91
Pb	125 ± 10	119 ± 17.77	82-105

The CRM031-040 (Sewage sludge standard reference material) was analysed using ICP-MS in duplicates and values indicate mean ± sd for each of the elements.

B.1.2. ICP-MS determined 'total' metals

Before the start of the field experiment, soil samples from the experimental site, dewatered biosolids from WWSP and composted biosolids from Pinegro were taken and the heavy metals were extracted with HNO_3/H_2O_2 and analysed using ICP-MS each in duplicate.

Table B-2 below shows that Cu and Zn in dewatered biosolids were higher than the levels found in composted biosolids, whereas Fe and Ni were higher in composted biosolids. The concentrations of Pb were relatively similar in both biosolids.

The levels of most of the heavy metals found in the soil were significantly lower than the levels found in both biosolids types with the exception of Fe (see also Appendix D Table D-1 to D-4).

Table B-2 ICP-MS determined concentrations of 'total' metals and micronutrients in soil, dewatered biosolids and composted biosolids (expressed in mg/kg)

Analytes	Soil	Dewatered biosolids	Composted biosolids
Cu	9 ± 1	416.0 ± 0.1	138 ± 1
Zn	2.0 ± 0.3	519 ± 1	365 ± 45
Mn	134 ± 2	170 ± 26	319 ± 4
Fe	25896 ± 349	9399 ± 155	28576 ± 150
Ni	14.8 ± 0.7	22 ± 3	27.2 ± 0.1
Pb	10.1 ± 1	29.9 ± 0.4	25 ± 3

Table B-3 Results of HNO_3/H_2O_2 ICP-MS determined 'total' soil metals and micronutrients in dewatered biosolids amended canola and oats plots expressed in mg/kg, 2006.

Dewatered biosolids amended soils from canola plots.				
Biosolids rates (t/ha)	Cu	Zn	Mn	Fe
0	1.8 ± 0.4	10.1 ± 0.2	121 ± 2	20955 ± 3192
5	1.4 ± 0.7	9 ± 3	118 ± 3	17009 ± 777
25	6 ± 2	20 ± 5	132 ± 4	23595 ± 536
45	17.4 ± 0.4	27 ± 10	154 ± 4	28059 ± 202
65	16 ± 2	22 ± 6	137 ± 22	26150 ± 454
F	2.8 ± 2.0	13 ± 10	118 ± 10	17757 ±162
LSD $_{0.05}$	2.8***	10.7*	19*	2784***
Dewatered biosolids amended soils from oats plots.				
Biosolids rates (t/ha)	Cu	Zn	Mn	Fe
0	9.80 ± 0.01	13 ± 1	125 ± 3	17900 ± 290
5	11 ± 1	14 ± 2	123 ± 2	17125 ± 115
25	14 ± 1	21± 3	131 ± 2	22937 ± 1069
45	19 ± 1	27 ± 4	134 ± 6	23737 ± 512
65	18 ± 2	27 ± 3	131 ± 3	23033 ± 819
Fertilized	2.6 ± 0.6	16 ± 6	121 ± 3	17150 ± 650
LSD $_{0.05}$	2.2***	4.9***	7.1*	1257***

The superscripts ***, **, * and ns refers to significant treatment effects in ANOVA (F-test) at p < 0.001, p < 0.01, p < 0.05 and not significant respectively, LSD stands for least significant difference (t-test) between mean values at p<0.05 level. Values indicate means of triplicate measurements (n = 3).

Dewatered biosolids and composted biosolids amended soil samples were taken after harvesting canola and oats crops in the 2006 field experiment and analysed using ICP-MS. The analytical results obtained from ICP-MS analysis are presented in Table B-3 and Table B-4 below.

Significant increases in the concentrations of Cu, Zn, Mn and Fe following dewatered biosolids application were noted. Similarly, the concentration of Cu, Zn and Fe in composted biosolids treated canola and oats plots also showed significant increases due to biosolids loadings; however, Mn concentrations in composted biosolids treated plots was not significantly different from the unamended control plots.

Table B-4 Results of HNO_3/H_2O_2 extracted and ICP-MS determined 'total' metals and micronutrients in composted biosolids amended soils in the 2006 experiment

Composted biosolids amended soils from canola plots 2006, mg/kg				
Biosolids rates (t/ha)	Cu	Zn	Mn	Fe
0	10 ± 1	28 ± 8	121 ± 8	17900 ± 1360
10	11.4 ± 0.4	43 ± 6	128 ± 4	17625 ± 405
30	13 ± 1	44 ± 8	128 ± 6	23123 ± 1279
50	15 ± 1	46 ± 12	133 ± 5	23210 ± 1360
70	17 ± 1	65 ± 17	133 ± 2	23340 ± 1008
Fertilized	2.2 ± 0.4	18 ± 15	122 ± 2	17163 ± 757
$LSD_{0.05}$	1.5***	20.8*	ns	2380***
Composted biosolids amended soils from oats plots 2006, mg/kg				
Biosolids rates (t/ha)	Cu	Zn	Mn	Fe
0	2 ± 1	16 ± 9	120 ± 16	16457 ± 246
10	5 ± 2	30 ± 6	117 ± 1	17045 ± 615
30	8 ± 3	46 ±12	127 ± 3	22157 ± 540
50	12 ± 1	44 ± 6	124 ± 7	22990 ± 250
70	20 ± 3	63 ±15	132 ± 5	22475 ± 35
Fertilized	2.4 ±0.2	11 ± 4	116 ±10	17007 ± 916
$LSD_{0.05}$	3.7***	20**	ns	714***

The superscripts ***, **, * and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.001$, $p < 0.01$, $p < 0.05$ and not significant respectively, $LSD_{0.05}$ stands for least significant difference (t-test) between mean values at $p < 0.05$ level. Values indicate means of triplicate measurements (n = 3).

B.2. X-ray fluorescence spectrometry (XRF) analysis

X-ray fluorescence spectrometry (XRF) is the most widely used method for the routine analysis of geological samples, including common rocks, soils and sediments, when major and common trace element data are required (Potts, 1998; Basta and McGowen, 2004). It is a non destructive analytical technique used to determine the elemental composition of various materials (Potts, 2004). The advantages of using XRF for elemental analysis include: faster analysis, low cost per sample and good accuracy and precision (Hans *et al*., 1992). Since it requires no extraction procedure, the analyst does not rely on good recovery from extraction procedure.

Sample preparation required for XRF analysis is minimal compared to conventional analytical techniques. However, for solid samples, since particle size, composition, and element form may affect the analysis, a homogeneous sample is usually prepared for quantitative analysis. It allows simultaneous determination of most elements with the exception of those with atomic number below 8.

Wavelength dispersive XRF spectrometry technique uses a crystal to diffract the X-rays, as the ranges of angular positions are scanned using a proportional detector and is the technique most used in soil analysis (Alloway, 1995). Because of its unique power in measuring the elemental composition of silicates and sulfides, the XRF method is an important instrument for the analysis of independent reference data (Jones, 1982).

For high quality results, samples should be analysed as glass disks, to eliminate mineralogical and particle size effects. Pressed pellets can also be used (Ma and Li, 1989) even for silicate rock analysis (Longerich, 1995).

B.2.1. Analytical quality assurance

For XRF analysis of total metals in soil/biosolids and biosolids amended soils, external calibration curves for each of the metals were established by analysing eight soil standard reference materials (NCS DC 73319, NCS DC 73320, NCS DC 73321, NCS DC 73322, NCS DC 73323, NCSDC 73324 NCS DC 73325 NCS DC 73326). Table B-5 shows the measured and certified values of these standard reference materials.

To validate the established calibration curves, Till1 and Till3 soil standard reference materials obtained from Canada were treated as samples and analysed for Cu, Zn, Mn, Ni and Pb and Fe.

The percentage recoveries of each of the metals compared with the certified values are shown in Table B-6.

Table B-5 Total metal concentrations of eight soil standard reference materials with their percentage recovery analysed using X-ray Fluorescence spectrometry for the purpose of establishing external calibration curves (expressed in mg/kg).

Soil SRM	Cu	Zn	Mn	Ni	Pb	Fe
NCS 73319 Certified	21±2	680±25	1760±63	20.4±1.8	98±6	51900 ± 900
Measured	20	679	1730	22	80	52000
Recovery (%)	95.2	99.9	98.3	107.8	81.6	100.2
NCS 73320 Certified	16.3±0.9	42±3	510±16	19.4±1.3	20±3	35200±700
Measured	18	49	470	18	23	34000
Recovery (%)	110.4	116.7	92.2	92.8	115.0	96.6
NCS 73321 Certified	11.4±1.1	31±3	304±14	12±2	26±3	20000±500
Measured	12	33.7	290	13	21	20000
Recovery (%)	105.3	108.7	95.4	108.3	80.8	100.0
NCS 73322 Certified	40±3	210±13	1420±75	64±5	58±5	103000±1100
Measured	40	214	1400	63	78	104000
Recovery (%)	100.0	102	98.6	98.4	134.5	101.0
NCS 73323 Certified	144±6	494±25	1360±71	40±4	552±29	126200±1800
Measured	141	490	1340	43	546	127000
Recovery (%)	97.9	99.2	98.5	107.5	98.9	100.6
NCS 73324 Certified	390±14	97±6	1450±82	53±4	314±13	80900±1300
Measured	390	94	1500	52	323	83000
Recovery (%)	100.0	96.9	103.4	98.1	102.9	102.6
NCS 73325 Certified	97±6	142±11	1780±113	276±15	14±3	187600±3300
Measured	100	151	1820	276	11	187000
Recovery (%)	103.1	106.3	102.2	100.0	78.6	99.7
NCS 73326 Certified	24.3±1.2	68±4	650±23	31.5±1.8	21±2	44800±500
Measured	24	70	600	29	21	44000
Recovery (%)	98.8	102.9	92.3	92.1	100.0	98.2

Note: Data behind "±" indicates uncertainty, $U = t\alpha \times S/\sqrt{N}$, where $\alpha = 0.01$, S refers to standard deviations and N for number of data ($N > 8$). For the measured values the standard deviations of the calibrated metals were: Cu (± 4); Zn (± 5); Mn (± 10); and Ni (± 2); Pb (± 3) and Fe (± 0.6).

Table B-6 Results of total metal concentrations for two soil Standard Reference Materials Till 1 and Till 3 analysed using XRF and expressed in (mg/kg) for validating the analytical data.

Till1	Measured	Certified	Recovery%	Till3	Measured	Certified	Recovery %
Cu	44	47	93	Cu	20	22	93
Zn	90	98	91	Zn	54	56	97
Mn	1310	1420	92	Mn	451	520	87
Fe	43500	48100	91	Fe	25000	27800	93
Ni	20	24	84	Ni	39	39	101
Pb	21	22	97	Pb	-	-	-

Table B-6 shows the results obtained from XRF analysis of Till 1 and Till 3 soil certified reference materials for Cu, Zn, Mn, Fe, Ni and Pb together with their certified values. The recovery of each of the heavy metals was calculated based on the value for Till 1 and Till 3 [(measured value (μg/g)/ certified value of Till (μg/g)) X100]. The lowest values recovered were 84 and 87% were found for Ni in Till1 and for Mn in Till3 respectively. The recoveries for Cu, Zn, Mn and Ni ranged between 91 to 101% except for Pb values in Till3 which were below detection limit. There were no significant differences between the measured and certified values ($p > 0.05$, pair wise t-test).

B.2.2. X-ray fluorescence determined total metals

Results of XRF analysis for soil, dewatered biosolids and composted biosolids showed that Cu and Zn levels in dewatered biosolids were considerably higher than the corresponding concentrations found in soil and composted biosolids, whereas the level of Mn found in soil and composted biosolids was slightly higher than the levels recorded in dewatered biosolids. The level of Fe found in dewatered biosolids was lower than the concentrations determined in soil and composted biosolids. Concentrations of Pb in composted biosolids were significantly higher than the levels found in soil and dewatered biosolids (Table B-7). Higher levels of Mn and Pb observed in the composted biosolids could be due to composting processes which increases concentrations of heavy metal (García et al., 1990; Ciavatta et al., 1993) because of microbial degradation of part of the organic matter and loss of volatile solids (Smith and Hall, 1991). Soil can also be considered as a source of heavy metals in both green waste and source segregated waste and would be accountable for increasing the metal concentrations of compost above quality limits (Fricke and Vogtmann, 1994).

Zn and Pb in most cases present in the largest amounts in source and mechanically-segregated municipal solid waste compost and green waste composts (Zennaro *et al*., 2005). The concentration of Pb in compost from mechanical-sorting municipal solid waste may be higher than the total Pb concentration measured in sewage sludge (Smith, 2009).

Table B-7 X-ray fluorescence determined concentrations of total heavy metal in soil, dewatered and composted biosolids expressed in mg/kg

Analytes	Soil	Dewatered biosolids	Composted biosolids
Cu	17.0 ± 0.8	648 ± 1	210 ± 4
Zn	37 ± 2	1062 ± 1	813 ± 8
Mn	246 ± 2	213 ± 1	299 ± 6
Fe	26000 ± 1000	14000 ± 100	25000 ± 1000
Ni	23.3 ± 0.4	27.17 ± 0.01	21.2 ± 0.4
Pb	11.4 ± 0.2	28 ± 3	47 ± 1

Values indicate mean ± standard deviations of triplicate measurements

Substantial increases in the concentrations of XRF determined total Cu, Zn and Mn residuals were also observed in plots treated with dewatered biosolids, however levels of Fe in canola plots and Mn in oats plots treated with dewatered biosolids were not significantly different from the control plots. Also, significant increases in the levels of Cu, Zn, Mn and Fe residuals following composted biosolids applications were also observed, however Fe did not show significant increase in composted biosolids treated oat plots (Table B-8 and Table B-9).

Table B-8 Concentrations of total metals determined using WD-XRF spectrometry in dewatered biosolids amended soil samples taken from canola plots in 2006 field experiments (mg/kg)

Biosolids rates (t/ha)	Cu	Zn	Mn	Fe
Dewatered biosolids amended soils from canola plots , mg/kg				
0	14 ± 2	30 ± 1	192 ± 6	30100 ± 4400
5	15 ± 1	33 ± 1	196 ± 3	26000 ± 1000
25	19 ± 1	37 ± 1	194 ± 5	31600 ± 400
45	26 ± 3	51 ± 4	204 ± 4	32500 ± 300
65	30 ± 2	55 ± 1	210 ± 10	32000 ± 1000
F	16 ± 1	30 ± 1	194 ± 3	26000 ± 1400
$LSD_{0.05}$	3.1***	3.8***	11.3*	ns
Dewatered biosolids amended soils from oats plots , mg/kg				
Biosolids rates (t/ha)	Cu	Zn	Mn	Fe
0	14 ± 1	31 ± 1	194 ± 6	26000 ± 1000
5	15 ± 3	32 ± 2	196 ± 9	26000 ± 2000
25	21 ± 3	42 ± 7	199 ± 3	32000 ± 2000
45	25 ± 2	46 ± 2	197 ± 9	33000 ± 1000
65	25 ± 1	47 ± 5	197 ± 7	32000 ± 1000
Fertilized	15 ± 2	31 ± 1	201 ± 4	26500 ± 1000
$LSD_{0.05}$	4.14***	8.27**	ns	2700***

The superscripts ***, **, * and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.001$, $p < 0.01$, $p < 0.05$ and not significant respectively, LSD stands for least significant difference (t-test) between mean values at $p<0.05$ level. Values indicate means of triplicate measurements ($n = 3$).

Table B-9 Concentrations of total metals and micronutrients determined using WD-XRF spectrometry in composted biosolids amended soil samples taken from canola plots in 2006 field experiments

Biosolids rates (t/ha)	Cu	Zn	Mn	Fe
Composted biosolids amended soils from canola plots (mg/kg)				
0	15 ± 2	33 ± 1	200 ± 1	25000 ± 1000
10	15 ± 3	34 ± 4	193.60 ± 0.01	26000 ± 1000
30	18 ± 2	44 ± 4	199 ± 2	31000 ± 2000
50	23.6 ± 0.3	55 ± 4	201 ± 4	31000 ± 1000
70	24 ± 1	59 ± 2	206 ± 2	32000 ± 1000
Fertilized	14 ± 1	30 ± 1	199 ± 8	26000 ± 1000
LSD 0.05	3.14***	7***	4.5**	1900***
Composted biosolids amended soils from oats plots 2006 (mg/kg)				
Biosolids rates (t/ha)	Cu	Zn	Mn	Fe
0	17 ± 3	38 ± 3	235 ± 35	30600 ± 4500
10	19 ± 1	44 ± 2	217 ± 23	29000 ± 4000
30	24 ± 3	60 ± 13	228 ± 31	41000 ± 1000
50	28 ± 3	73 ± 15	229 ± 27	41800 ± 200
70	34 ± 8	91 ± 24	232 ± 32	40400 ± 600
Fertilized	16 ± 4	36 ± 5	224 ± 39	30000 ± 5000
$LSD_{0.05}$	5.8***	22***	ns	4600***

The superscripts ***, **, * and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.001$, $p < 0.01$, $p < 0.05$ and not significant respectively, $LSD_{0.05}$ stands for least significant difference (t-test) between mean values at $p < 0.05$ level. Values indicate means of triplicate measurements (n = 3).

B.3. Residuals of metals in biosolids amended soil after growing canola and oats and comparisons of ICP-MS and XRF techniques

B.3.1. Metal residues in biosolids amended soil

Figures B-1 to Figure B-5 show the change in residual “total” metal concentration for metal as measured by XRF and ICPMS, at each application rate, for each type of biosolids and for each crop. When the results of the two techniques are compared, a number of observations can be made.

Both methods showed that the increase in the copper in canola plots treated with dewatered biosolids was substantially greater than the values recorded in the plots treated with composted biosolids, However Cu residuals recorded in the oat plots treated with dewatered and composted biosolids were relatively similar except for a slightly elevated Cu concentrations observed in the plot treated with 70 t/ha.

On average, the results for copper using XRF were 47 % in dewatered biosolids treated plots and 50 % more in the composted treated plots that those measured using ICP-MS analysis (Fig B-1).

Despite the high levels of Zn found in dewatered biosolids, the concentrations of Zn residues observed in composted biosolids amended soil was significantly greater than Zn levels found in dewatered biosolids treated canola and oats plots. It is expected that greater proportion of Zn found in dewatered biosolids would be in the exchangeable and water soluble forms and during watering the canola and oats crops a substantial amount of water soluble and exchangeable forms of Zn may have leached down beyond the 10 cm sampling depth.

Concentrations of Zn recorded from XRF analysis was significantly higher than the Zn levels recorded from ICP-MS analysis. XRF on average gives 53 % more Zn concentrations in dewatered biosolids treated plots than the corresponding Zn values recorded from ICP-MS technique.

The results from the composted biosolids treated plots were more variable with the XRF results from the oats plots being 37 % greater than those recorded from ICP-MS. However, in composted biosolids treated canola plots, the residuals of Zn determined using XRF and ICP-MS were similar. Using either technique, it can be seen that concentrations of Zn residues in biosolids amended soil significantly increased following increased dewatered and composted biosolids application rates (Fig B-2).

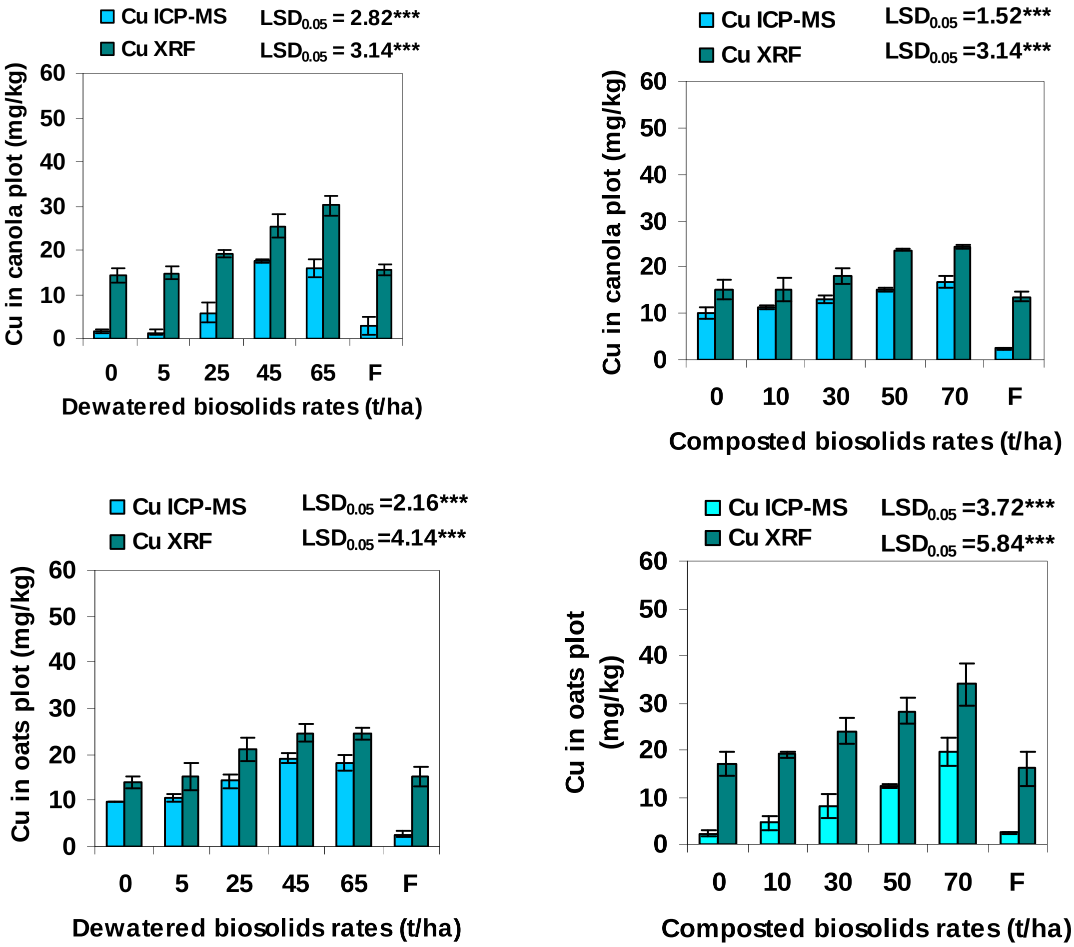


Figure B-1 A comparison between ICP-MS and X-Ray Fluorescence Spectrometry (XRF) determined total Cu in dewatered and composted biosolids amended soils sampled from canola and oats plots in 2006 field experiment. LSD $_{0.05}$ refers to the least significant difference (t-test) between the mean values of Cu at 5 % probability level. The error bars indicate standard deviations of triplicate measurements, whereas, *** refers to significant treatments effects in ANOVA (F-test) at $p < 0.001$ level.

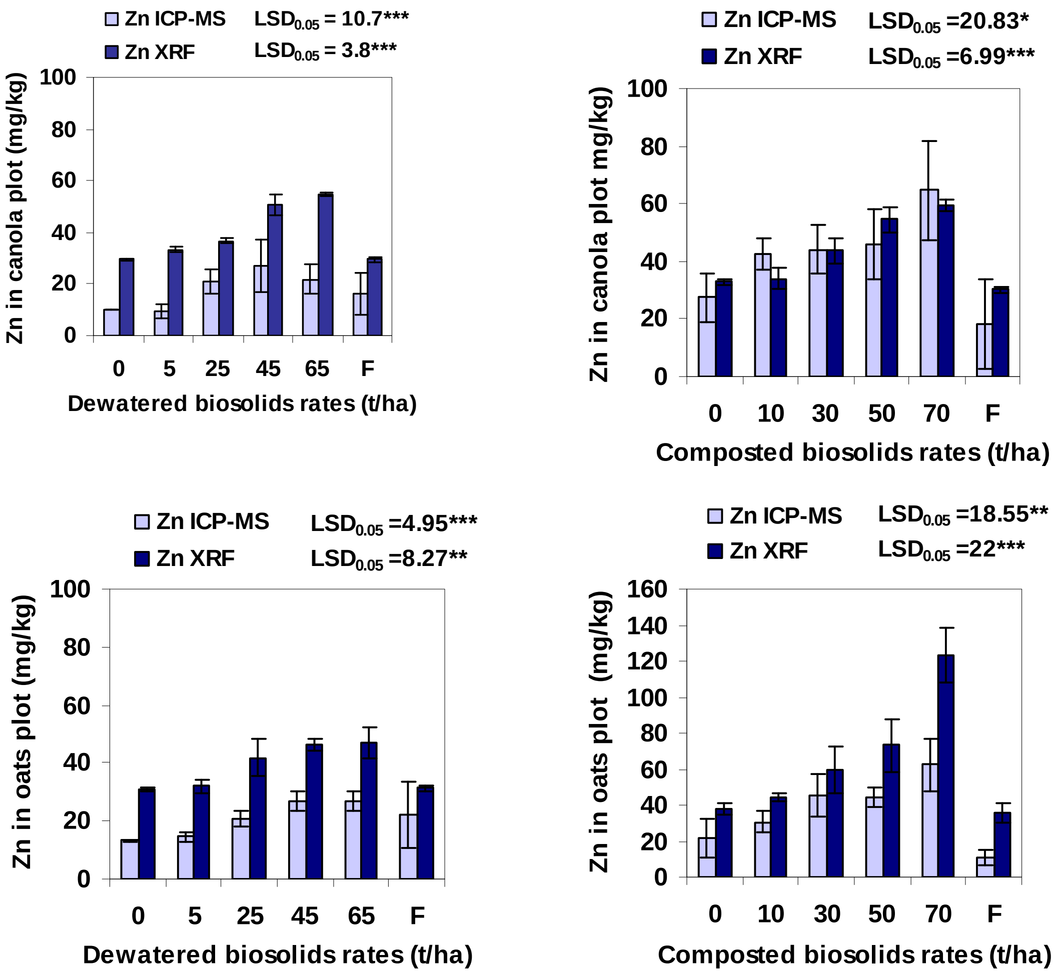


Figure B-2 A comparison between ICP-MS and X-Ray Fluorescence Spectrometry (XRF) determined total Zn in dewatered and composted biosolids amended soils sampled from canola and oats plots in 2006 field experiment. LSD $_{0.05}$ refers to the least significant difference (t-test) between the mean values of Zn at 5 % probability level. The error bars indicate standard deviations of triplicate measurements, whereas,*,** and *** refers to significant treatments effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$ and $p < 0.001$ levels respectively.

The levels of Mn residues observed in canola plots treated with dewatered and composted biosolids were relatively similar ; however, as expected Mn levels in composted biosolids treated oats plots was slightly higher than the levels found in oats plots treated with dewatered biosolids, since Mn concentrations in composted biosolids was higher than the levels found in dewatered biosolids. Concentrations of Mn recorded from XRF analysis was on average higher by 34 % in plots treated with dewatered biosolids than the corresponding results obtained from ICP-MS analysis; in composted biosolids treated plots, XRF gave 41 % more Mn than the results recorded from ICP-MS analysis (Fig B-3).

Fe residues observed in dewatered biosolids treated canola and oats plots were similar. XRF determined concentrations of Fe in dewatered biosolids amended plots was higher than the levels observed in ICP-MS analysis by 27 %, likewise XRF gave 36 % more Fe concentrations in composted biosolids treated plots than ICP-MS results (Fig B-4).

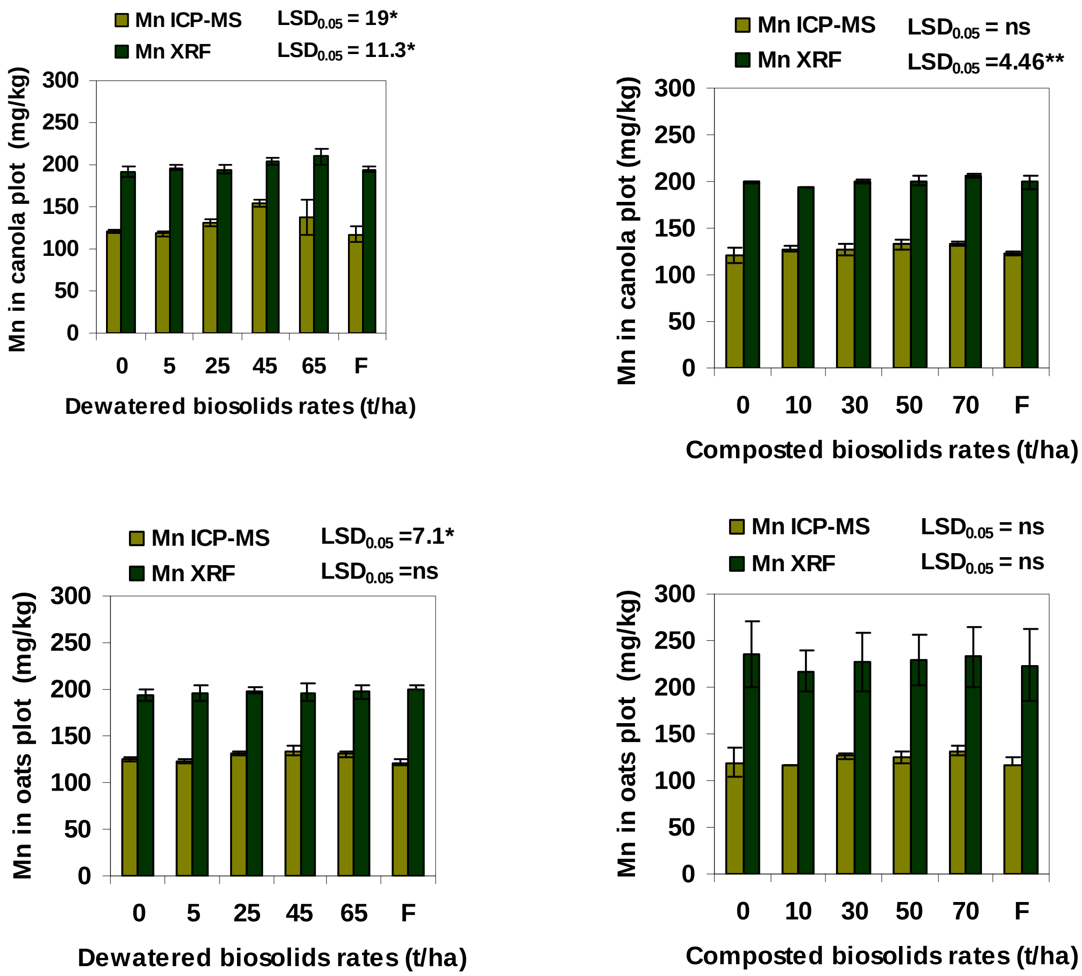


Figure B-3 A comparison between ICP-MS and X-Ray Fluorescence Spectrometry (XRF) determined total Mn in dewatered and composted biosolids amended soils sampled from canola and oats plots in 2006 field experiment. LSD $_{0.05}$ refers to the least significant difference (t-test) between the mean values of Mn at 5 % probability level. The error bars indicate standard deviations of triplicate measurements, whereas,*,** and ns refers to significant treatments effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$ and not significant respectively.

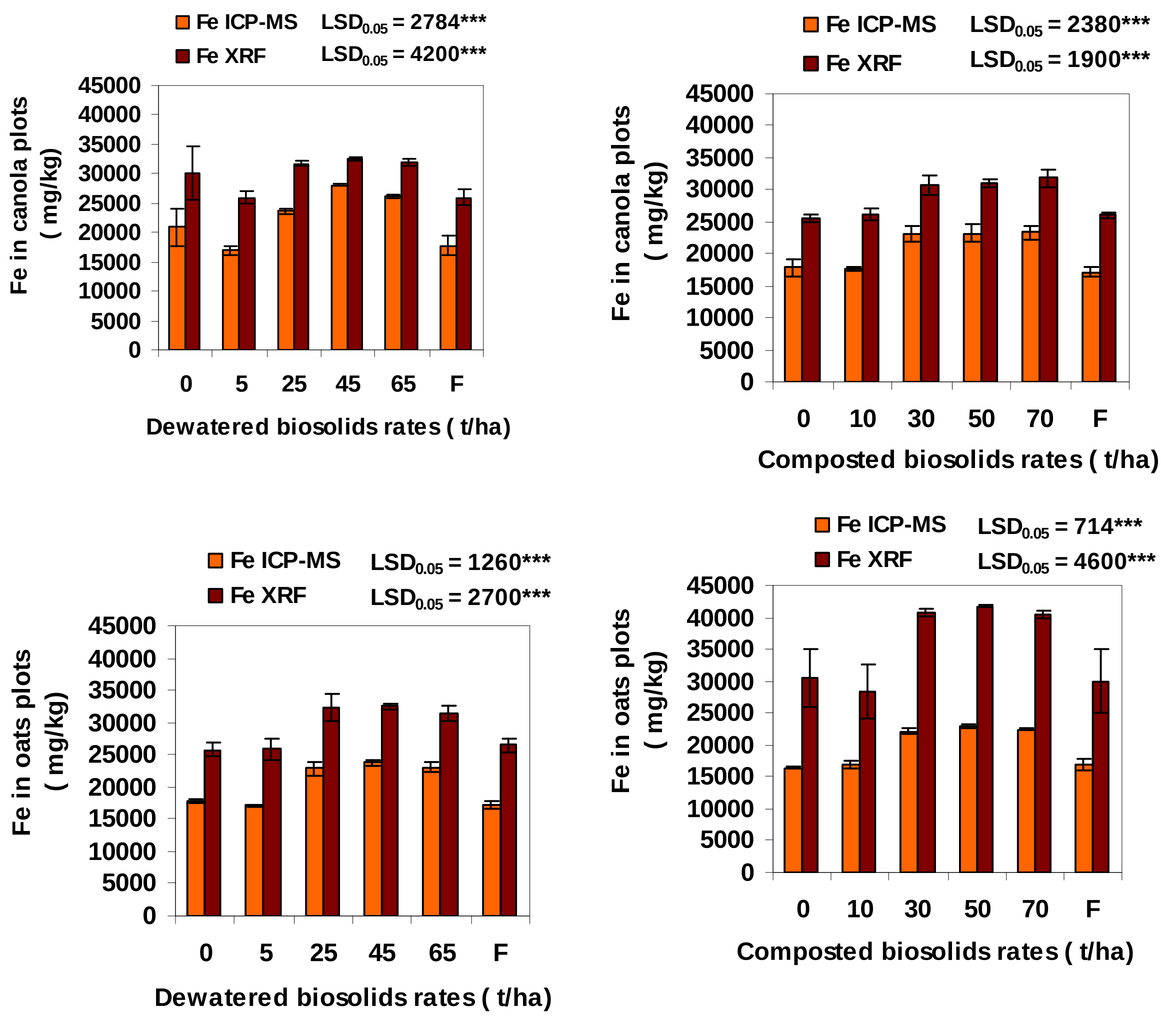


Figure B-4 A comparison between ICP-MS and X-Ray Fluorescence Spectrometry (XRF) determined total Fe in dewatered and composted biosolids amended soils sampled from canola and oats plots in 2006 field experiment. LSD $_{0.05}$ refers to the least significant difference (t-test) between the mean values of Fe at 5 % probability level. The error bars indicate standard deviations of triplicate measurements, whereas,* and *** refers to significant treatments effects in (ANOVA, F-test) at $p < 0.05$ and $p < 0.001$ respectively.

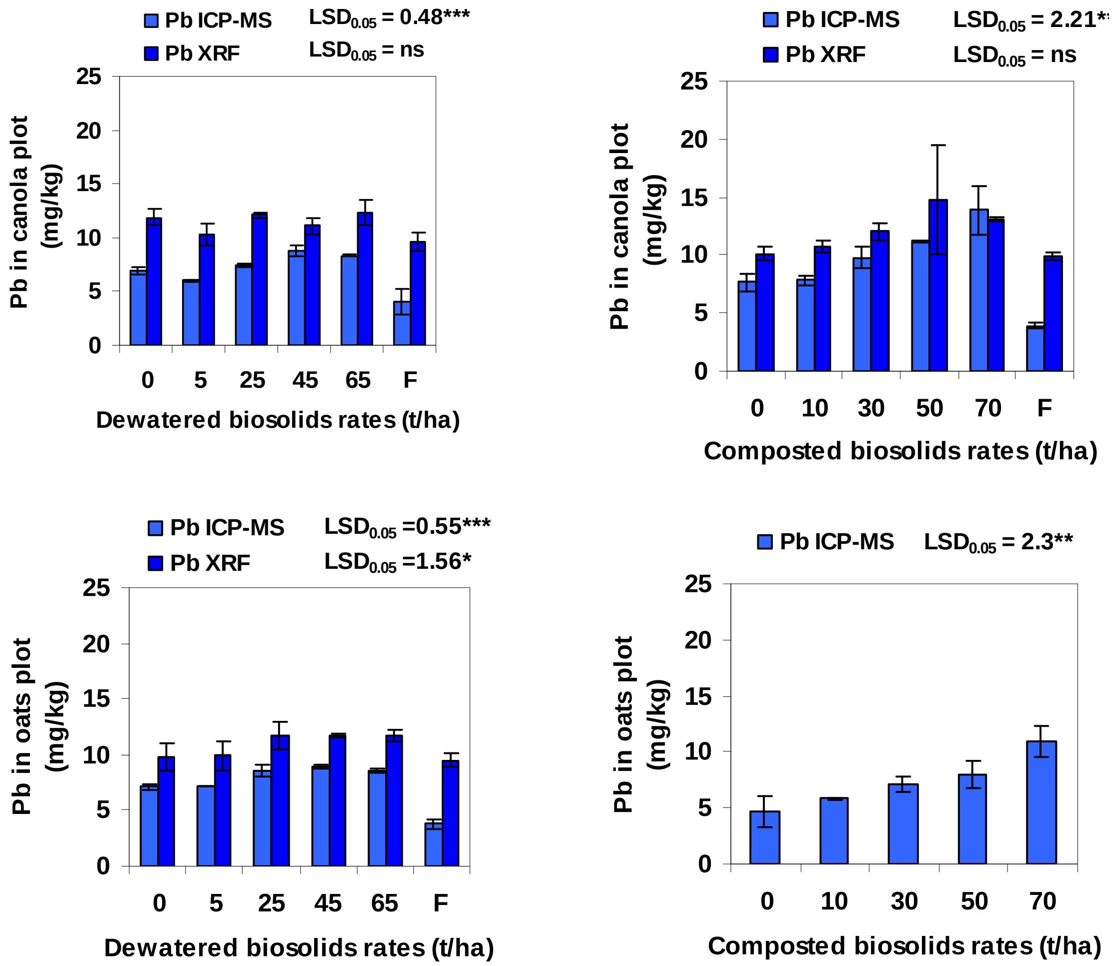


Figure B-5 A comparison between ICP-MS and X-Ray Fluorescence Spectrometry (XRF) determined total Pb in dewatered and composted biosolids amended soils sampled from canola and oats plots in 2006 field experiment. LSD $_{0.05}$ refers to the least significant difference mean separation (t-test) between the mean values of Pb at 5 % probability level. The error bars indicate standard deviations of triplicate measurements, whereas,*, **, *** and ns refers to significant treatments effects in (ANOVA, F-test) at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively.

Pb values obtained from XRF analysis of dewatered biosolids amended soil was 31 % higher than the values observed using ICP-MS, whereas in composted biosolids treated canola plots, concentrations of Pb determined using XRF was slightly higher by 6 % than ICP-MS recorded Pb values. The level of Pb in oats plots treated with dewatered biosolids and in canola plots treated with composted biosolids increased as dewatered and composted biosolids application rates increased. The concentrations of XRF determined Pb in composted biosolids treated oats plots was not retrieved from the instrument, only ICP-MS determined Pb concentration in oats plots treated with composted biosolids was presented (Figure B-5).

Similar with this findings Chander *et al*. (2008) compared the relationship between aqua regia, HNO_3 pressure digested (ICP-AES) and XRF determined heavy metals in contaminated soil and reported that the aqua regia digestible fraction of Pb, Cr, Cu, Zn and Ni reached on average 64 % of the XRF-detectable content whereas the pressure accelerated HNO3-digestible fraction of the five heavy metals was on average 71% of the XRF-detectable content.

Wilson *et al*. (1995) analysed potentially contaminated soils for Cu, Ni and Zn contents using ICP-OES and XRF techniques and suggested that results obtained by XRF were slightly higher than those obtained by ICP-OES, due to incomplete digestion of the samples in preparation for analysis by ICP-OES.

Makinen *et al*. (2005) also analysed As, Cu and Cr in soils polluted by chromated copper arsenate using XRF and FAAS. Their findings showed that FAAS and intrusive laboratory measurements with XRF showed good correlation between As and Cu results.

Duane *et al*. (1995) analysed heavy metals in soil samples collected from a disused industrial area so as to evaluate the performance of a mobile laboratory equipped with ICP-MS (aqua regia digested) and compare the results with fixed-lab based ICP-AES/GFAAS and ED-XRF. From the analysis of CRM-320 reference material, it was reported that except Cr, Zn and Pb both the XRF and ICP-MS produced similar results.

B.4. Correlations between ICP-MS (HNO_3/H_2O_2 extractable) and XRF determined total metals in biosolids amended soil

Significant positive correlations were observed between the two methods (ICP-MS and XRF) determined Cu, Zn, Mn, Pb and Fe concentrations in both canola and oats plots treated with dewatered and composted biosolids as shown in Fig B-6 – Fig B-8.

Over all, the heavy metals determined using ICP-MS and XRF in dewatered and composted biosolids amended soil were quite significantly correlated (Appendix E Table E-1 and E-2). Compared with XRF, the ICP-MS underestimated the total concentrations of heavy metals on average by 52 % for Cu, 64 % for Zn, 62 % for Mn and 73 % for Fe respectively. This would be due to the use of an HNO_3/H_2O_2 extraction solution which would not have provided complete digestion of the biosolids amended soil samples for the ICP-MS analysis.

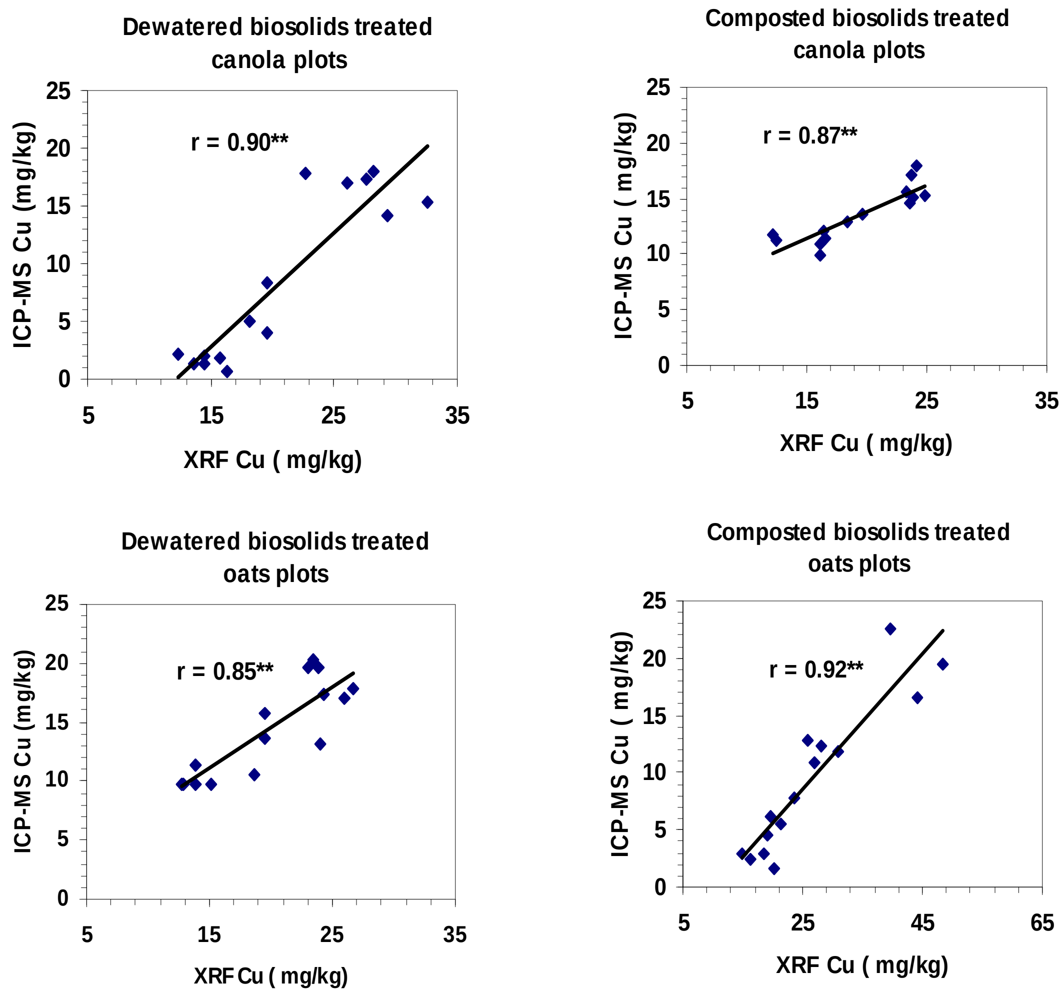


Figure B-6 Pearson correlation between XRF and ICP-MS determined concentrations of Cu in dewatered and composted biosolids amended canola and oats plots in 2006. The superscripts * and ** refer to significance for Pearson's correlation coefficient at $p < 0.05$ and $p < 0.01$ level respectively

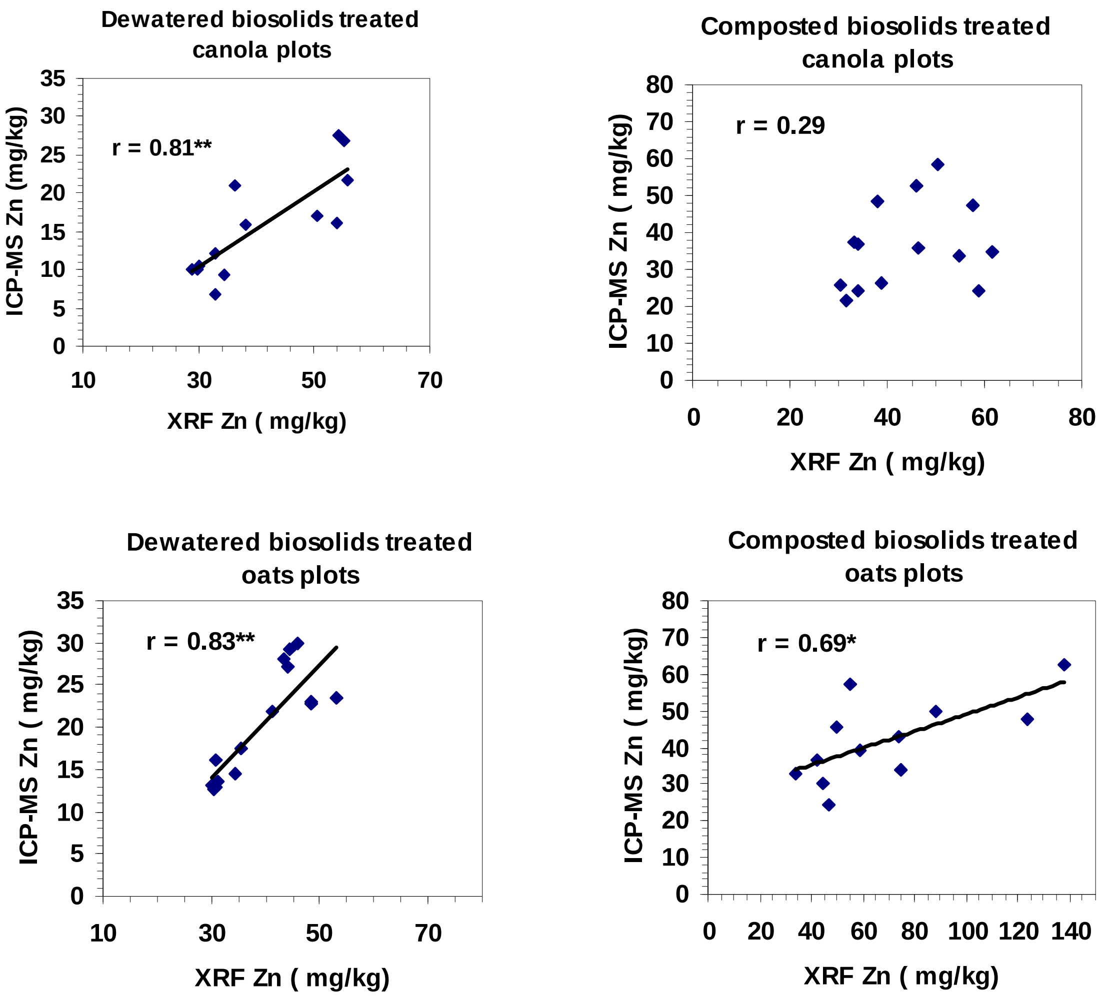


Figure B-7 Pearson correlations between XRF and ICP-MS determined concentrations of Zn in dewatered and composted biosolids amended canola and oats plots, 2006. The superscripts * and ** refer to significance for Pearson's correlation coefficient at $p < 0.05$ and $p < 0.01$ level respectively

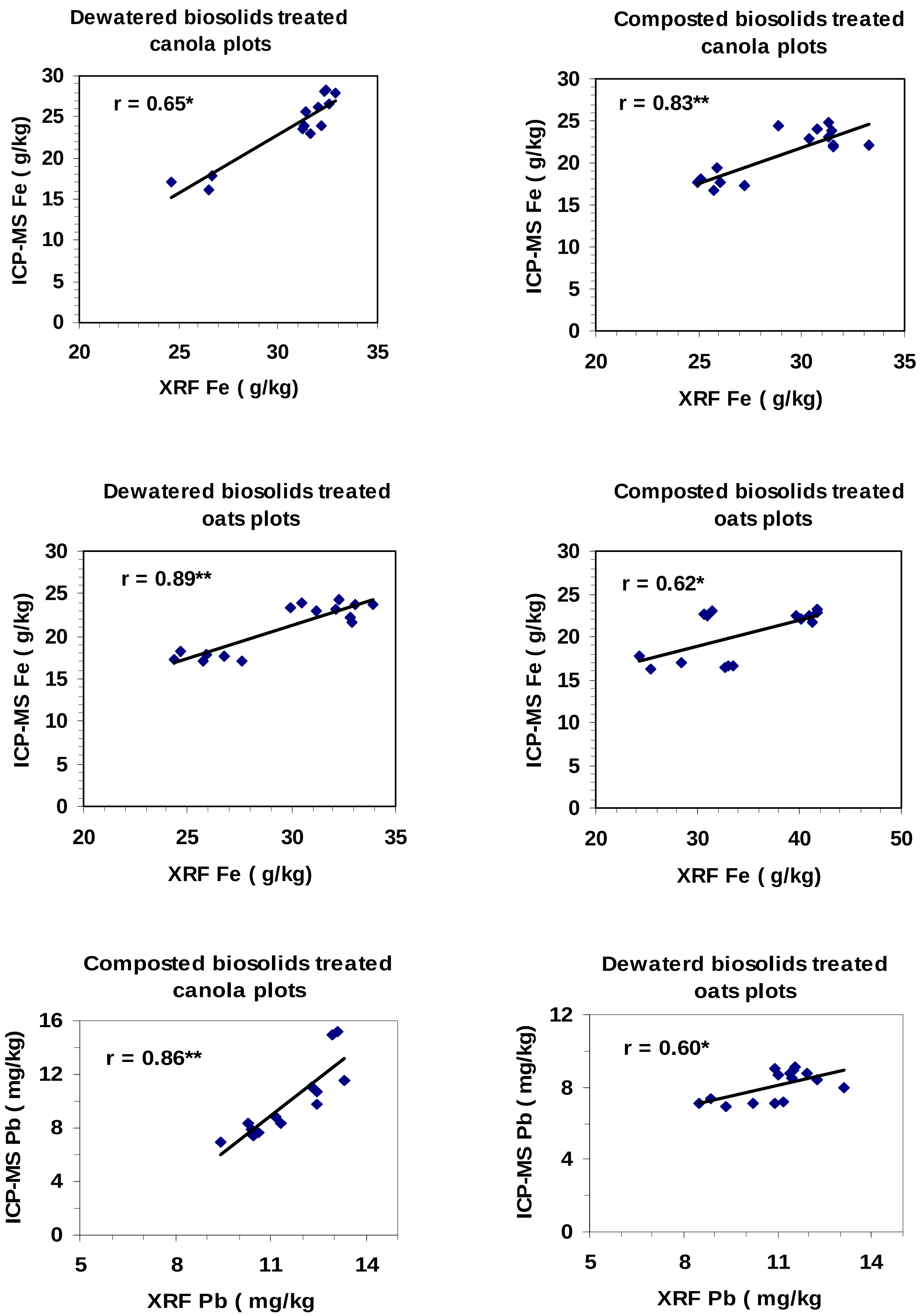


Figure B-8 Pearson correlations between XRF and ICP-MS determined Fe and Pb in dewatered biosolids and composted biosolids amended canola and oats plots, 2006.

B.5. Relative extractability of metals in biosolids amended soil

The relative extractability (HNO_3/H_2O_2) of the 'total' metals analysed using ICP-MS are expressed as percent of XRF total for biosolids amended soils from canola and oats plots as shown in Table B-10 and B-11 respectively. For comparison purposes, the ratio of ICP-MS to XRF heavy metal values for the background soil, dewatered and composted biosolids were also presented.

The data for the ratio of ICP-MS to XRF determined total metals for Cu, Zn, Mn, Pb and Fe showed some variations following dewatered biosolids application rates in canola and oats plots. However, the results indicated that increases in the percentage values of the ratio of ICP-MS to XRF determined total heavy metals were observed due to dewatered biosolids application rates (Table B-10).

Likewise, a slight increase in the ratio of ICP-MS to XRF percent values for Cu, Zn, Mn, Pb and Fe following composted biosolids applications were also noted in both canola and oats plots (Table B-11).

As expected these slight increases in the ratio of ICP-MS to XRF percent values for Cu, Zn, Mn, Pb and Fe following dewatered and composted biosolids application rates were probably due to the increased organic matter added to the amended soil due to biosolids application rates. Hence, during the sample preparation step HNO_3/H_2O_2 digestion had stronger and more aggressive effect on samples with high organic matter content, these might have contributed to increases in the values for the ratio of ICP-MS to XRF determined heavy metals values at various biosolids application rates.

Table B-10 Comparison between XRF total and ICP-MS (HNO_3/H_2O_2 extracted) total metals and micronutrients in dewatered and composted biosolids amended soils from canola plots after first year crop harvest in 2006 field experiment (ICP-MS values expressed as percent of XRF total).

Soil samples taken from dewatered biosolids treated canola plots					
Dewatered biosolids rates	Cu	Zn	Mn	Pb	Fe
	% ICP/ XRF	% ICP/XRF	% ICP/XRF	% ICP/XRF	% ICP/XRF
0	12	34	63	58	70
5	9	28	60	59	66
25	30	57	68	61	74
45	68	54	76	79	86
65	53	40	66	68	82
Soil[a]	53	5	54	89	100
Dewatered biosolids[b]	64	49	80	107	67
Soil samples taken from composted biosolids treated canola plots					
Composted biosolids rates	Cu	Zn	Mn	Pb	Fe
	% ICP/ XRF	% ICP/XRF	% ICP/XRF	% ICP/XRF	% ICP/XRF
0	67	84	61	76	70
10	76	125	66	73	68
30	71	101	64	81	76
50	64	84	66	76	75
70	69	109	65	106	73
Soil[a]	53	5	54	89	100
Composted biosolids[c]	66	45	107	53	114

The superscript ‘a’ refers to the ratio of ICP-MS to XRF determined concentrations of heavy metals in soil (back ground levels before the start of the experiment), whereas ‘b’ and ‘c’ refer to the ratio of ICP-MS to XRF total heavy metal concentrations in the dewatered and composted biosolids respectively.

Over all, for the analysis of total metals in soil and biosolids, the findings of this experiment showed that the XRF technique was rapid, more efficient and produced highly reproducible and accurate results than the ICP-MS technique and hence for this study, XRF was chosen for the analysis of total heavy metals in soil and biosolids and biosolids amended soil.

B.6. Conclusion

Levels of Cu, Zn and Fe determined using both ICP-MS and XRF in biosolids amended soil increased as both types of biosolids application rates increased, with XRF determined total heavy metals being significantly higher than their corresponding ICP-MS determined values.

Significant positive correlation between ICP-MS and XRF determined total Cu, Zn, Fe and Pb were observed for soil samples taken from dewatered and composted biosolids treated canola and oats plots with the exception of Zn in composted biosolids treated canola plots.

Slight increments in the values of the ratio of ICP-MS to XRF values for most of the heavy metals at higher biosolids application rates (45 and 65 t/ha dewatered and 50 and 70 t/ha composted biosolids rates) were observed, suggesting that HNO_3/H_2O_2 extractable fractions of the heavy metals were to some extent affected by the organic matter content of the amended soil.

Data from ICP-MS determined soil Cu, Zn, Mn, Fe and Pb values at various dewatered bioasolids and composted biosolids application rates in canola and oats plots showed some variations. These variations were expected to occur due to the increased organic matter added to the soil from the various biosolids application rates. Thus, during the sample digestion procedure HNO_3/H_2O_2 had stronger and more aggressive effect on samples with high organic matter content than samples with low organic matter content, hence this might have contributed to the variations in the ICP-MS values expressed as percent of XRF determined total heavy metals.

From the findings of this study, it can be concluded that for higher recoveries of metals in the biosolids amended soil, the use of alternative acids in the digestion (eg $HClO_4$ and HF) could be employed for the ICP-MS analysis.

XRF is a better option for total metal analysis particularly for soils analysis due to its shorter time of sample preparations and ease of instrument operation. In XRF analysis, sample preparation does not need pre-concentration steps and this saves time and avoids the risks from contamination, it does not use chemical reagents thus significantly reducing analytical costs.

In addition to this, the smaller the standard deviations and reproducibility and consistency of the results, XRF would be the preferred option for routine laboratory analysis of soil samples.

APPENDIX C

Concentrations of total elements in biosolids amended soils determined using x-ray fluorescence spectrometry, 2006

Table C-1. X-ray fluorescence determined concentrations of total elements in dewatered biosolids amended soil in canola plots, 2006 field experiment.

Analytes	Dewatered biosolids application rates (t/ha)					
	0	5	25	45	65	Fertilized	LSD $_{0.05}$
P (µg/g)	647	797	876	1054	1290	769.9	93***
S (µg/g)	533	573	606	769	836	633.3	118***
Mn (µg/g)	192	196.2	194.3	203.5	209.5	194.3	11.27*
Cu (µg/g)	14.16	14.8	19.11	25.46	30.04	15.51	3.14***
Zn (µg/g)	29.55	33.44	36.78	50.7	54.65	29.55	3.8***
Cr (µg/g)	20	25	27	30.7	38.5	10.4	ns
Ni (µg/g)	26.02	25.17	24.48	26.83	28.55	24.93	ns
Pb (µg/g)	11.86	10.34	12.15	11.09	12.3	9.66	ns
Cl (µg/g)	145.1	101.4	71.4	79.6	82.8	71.11	49.7*
Fe (%)	3.01	2.59	3.16	3.25	3.2	2.59	ns
C (%)	5.54	6.14	5.89	6.11	6.24	6.14	0.41*
Ca (%)	0.31	0.33	0.29	0.38	0.35	0.32	ns
Al (%)	5.86	6.72	5.98	6.27	6.05	6.77	ns
Na (%)	0.23	0.20	0.20	0.20	0.21	0.19	0.02*
K (%)	0.93	0.93	0.93	0.93	0.93	0.93	ns
Mg (%)	0.57	0.49	0.41	0.41	0.38	0.35	0.074**

Table C-2. X-ray fluorescence determined concentrations of total elements in composted biosolids amended soil in canola plots, 2006 field experiment (t/ha)

Analytes							
	0	10	30	50	70	Fertilized plot	LSD $_{0.05}$
P (µg/g)	719.17	947.03	1362.00	1884.00	2231.67	780.30	253.6***
S (µg/g)	576.53	563.75	614.3	737.63	753.83	597.70	74.8***
Mn (µg/g)	199.5	193.60	199.35	200.97	205.80	199.40	4.46**
Cu (µg/g)	14.91	14.99	18.14	23.60	24.24	13.57	3.14***
Zn (µg/g)	32.92	34.01	43.78	54.66	59.33	30.38	6.99***
Cr (µg/g)	7.51	19.80	30.15	16.93	24.99	21.31	7.79**
Ni (µg/g)	25.65	25.33	23.91	24.39	25.32	25.02	1.12*
Pb (µg/g)	10.10	10.72	12.03	14.80	13.12	9.91	ns
Cl (µg/g)	68.48	76.39	71.57	74.89	75.33	71.49	ns
Fe (%)	2.55	2.61	3.06	3.11	3.18	2.61	0.19***
C (%)	6.61	6.72	6.39	6.58	6.76	6.64	ns
Ca (%)	0.35	0.42	0.44	0.48	0.58	0.42	ns
Al (%)	6.15	6.06	5.81	5.81	5.83	6.02	0.22*
Na (%)	0.19	0.19	0.19	0.20	0.19	0.19	ns
K (%)	0.96	0.95	0.92	0.93	0.93	0.94	0.022*
Mg (%)	0.32	0.31	0.31	0.33	0.33	0.29	ns
F	250.43	250.8	331.63	319.03	232.55	241.15	ns
Mo	3.11	3.19	3.12	2.89	2.93	3.37	ns

The superscripts ***, **, * and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.001$, $p < 0.01$, $p < 0.05$ and not significant respectively, LSD stands for least significant difference (t-test) between mean values at $p < 0.05$ level.

Table C-3. X-ray fluorescence determined concentrations of total elements in dewatered biosolids amended soil in oats plots in 2006 field experiment.

Analytes	Dewatered biosolids application rates (t/ha)						
	0	5	25	45	65	Fertilized plot	LSD $_{0.05}$
P (μg/g)	707.7	780.33	942.23	1093.8	1192.67	740.53	234.6**
S (μg/g)	531.57	506.67	580.30	753.50	673.37	551.43	136.4**
Mn (μg/g)	193.50	195.67	198.60	196.80	197.10	200.67	ns
Cu (μg/g)	13.92	15.11	21.05	24.55	24.58	15.42	4.14***
Zn (μg/g)	30.67	32.02	41.73	46.15	47.07	31.22	8.27**
Cr (μg/g)	13.24	20.42	21.06	24.21	19.03	18.95	ns
Ni (μg/g)	23.73	24.98	25.32	27.79	25.10	25.08	ns
Pb (μg/g)	9.80	9.88	11.68	11.67	11.70	9.53	1.56*
Cl (μg/g)	68.82	74.79	68.25	73.44	71.66	68.26	ns
Fe (%)	2.58	2.59	3.23	3.25	3.15	2.65	0.27***
C (%)	6.50	6.70	5.80	5.90	6.50	6.61	0.55*
Ca (%)	0.34	0.31	0.32	0.33	0.30	0.32	ns
Al (%)	3.18	3.17	3.59	3.92	3.53	2.88	ns

Table C-4. X-ray fluorescence determined concentrations of total elements in composted biosolids amended soil in oats plots in 2006 field experiment.

Analytes	Composted biosolids application rates (t/ha)						
	0	10	30	50	70	Fertilized plot	LSD $_{0.05}$
P (µg/g)	841.17	1261.50	1876.00	3131.00	5126.00	839.57	480***
S (µg/g)	665.23	684.50	557.47	845.80	999.10	592.43	254*
Mn (µg/g)	235.17	217.30	227.50	228.70	232.30	223.87	ns
Cu (µg/g)	17.12	19.12	23.98	28.27	44.02	15.99	5.84***
Zn (µg/g)	37.46	44.25	59.58	73.41	123.40	35.67	22***
Ni (µg/g)	29.13	28.92	30.18	31.65	29.49	29.35	ns
Cl (µg/g)	54.24	56.83	52.25	52.46	54.72	49.14	ns
Fe (%)	3.06	2.85	4.07	4.18	4.04	3.00	0.46***
C (%)	3.63	5.29	2.52	4.16	4.88	4.54	ns
Ca (%)	0.53	0.31	0.51	0.63	1.21	0.54	0.28***
Mg (%)	0.31	0.93	0.31	0.35	0.38	0.31	0.05***
K(%)	1.02	0.68	0.97	1.00	0.98	1.00	ns
Al (%)	6.26	6.05	5.86	5.93	5.59	6.20	ns

The superscripts * , **, ***, and ns refers to significant treatment effects in ANOVA at $p \leq 0.05$, $p < 0.01$ $p \leq 0.001$ and not significant, respectively. Values indicate means of triplicate measurements

Table C-5. X-ray fluorescence determined concentrations of total elements in dewatered biosolids amended soil oats plots in 2007 field experiment.

Analytes	Dewatered biosolids application rates (t/ha)						
	0	5	25	45	65	Fertilized plot	LSD $_{0.05}$
P (µg/g)	793.40	1041.63	1439.67	2046.67	2223.67	921.20	161***
S (µg/g)	288.13	352.57	506.75	646.73	713.33	352.03	79***
Mn (µg/g)	240.00	247.00	246.77	250.20	241.93	243.70	5.6**
Cu (µg/g)	18.42	22.48	29.74	46.81	49.13	19.27	7.2***
Zn (µg/g)	36.62	45.57	58.48	87.77	89.71	39.22	11***
Ni (µg/g)	35.68	36.86	34.85	38.21	36.62	36.48	1.01**
Cl (µg/g)	50.03	53.99	48.20	45.33	45.96	45.80	6.4*
Fe (%)	4.26	4.23	4.12	4.33	4.21	4.21	ns
C (%)	2.10	2.37	2.62	2.75	2.85	2.21	0.31**
Ca (%)	0.32	0.34	0.36	0.40	0.36	0.33	ns
Mg (%)	0.32	0.32	0.32	0.34	0.33	0.31	ns
K (%)	1.08	1.06	1.05	1.06	1.04	1.10	ns
Al (%)	6.50	6.34	6.31	6.59	6.45	6.47	ns

Table C-6. X-ray fluorescence determined concentrations of total elements in composted biosolids amended soil oats plots in 2007 field experiment.

Analytes	Composted biosolids application rates (t/ha)					
	0	10	30	50	70	Fertilized	$LSD_{0.05}$
P (µg/g)	714.00	1456.33	2450.33	4431.67	5698.67	910.23	327***
S (µg/g)	393.90	345.37	430.00	649.00	790.03	309.53	43.84***
Mn (µg/g)	187.07	249.60	248.27	258.83	273.53	240.57	16.02***
Cu (µg/g)	15.22	21.33	29.49	42.02	50.58	17.81	2.03***
Zn (µg/g)	31.89	52.29	70.70	112.03	138.50	38.17	9.9***
Ni (µg/g)	25.05	35.58	34.40	35.14	36.13	33.09	2.03***
Cl (µg/g)	52.01	45.19	41.72	42.65	42.56	43.05	4.74**
Fe (%)	3.09	4.12	4.02	4.21	4.44	4.03	0.23***
C (%)	5.53	2.30	2.69	2.83	3.16	2.28	0.26***
Ca (%)	0.32	0.48	0.55	0.73	0.86	0.43	0.14***
Mg (%)	0.30	0.32	0.33	0.38	0.40	0.29	0.02***
K (%)	0.92	1.06	1.05	1.06	1.06	1.04	0.036***
Na (%)	0.19	0.22	0.22	0.25	0.22	0.22	0.023**
Al (%)	5.86	6.22	6.05	5.87	5.80	6.16	0.25*

Table C-7. X-ray fluorescence determined concentrations of total elements in dewatered biosolids amended soil in canola plots in 2007 field experiment.

Analytes	Dewatered biosolids application rates (t/ha)						
	0	5	25	45	65	Fertilized plot	LSD $_{0.05}$
P (µg/g)	803.70	972.73	1497.00	1930.00	2035.33	853.33	310***
S (µg/g)	248.03	316.17	484.97	584.90	727.65	279.13	51.85***
Mn (µg/g)	242.10	241.67	241.17	241.07	238.10	246.33	ns
Cu (µg/g)	18.53	23.03	33.37	42.57	45.26	19.96	6.6***
Zn (µg/g)	40.08	45.40	63.27	73.85	81.46	40.72	11.5***
Ni (µg/g)	34.80	35.57	35.57	37.28	34.18	35.92	ns
Cl (µg/g)	40.64	42.50	46.07	44.62	48.80	42.43	3.7**
Fe (%)	4.13	4.18	4.15	4.19	4.16	4.28	ns
C (%)	2.06	2.18	2.57	2.71	2.68	2.09	0.26**
Ca (%)	0.35	0.34	0.39	0.40	0.34	0.39	ns
Mg (%)	0.31	0.31	0.32	0.32	0.31	0.31	ns
K (%)	1.07	1.06	1.05	1.04	1.02	1.08	ns
Na (%)	0.22	0.22	0.22	0.21	0.21	0.22	ns
Al (%)	6.38	6.34	6.37	6.37	6.22	6.43	ns

Table C-8. X-ray fluorescence determined concentrations of total heavy metals in composted biosolids amended soil in canola plots in 2007 field experiment.

Analytes	Composted biosolids application rates (t/ha)						
	0	10	30	50	70	Fertilized plot	LSD $_{0.05}$
P (µg/g)	831.10	1338.00	2291.33	3527.33	4389.33	862.10	355.5***
S (µg/g)	258.43	384.70	567.70	790.90	926.90	321.30	105.5***
Mn (µg/g)	242.40	238.15	251.40	262.00	267.30	235.73	9.2***
Cu (µg/g)	18.46	21.84	29.51	40.24	47.86	19.07	5.8***
Zn (µg/g)	40.73	51.33	70.78	97.93	121.43	39.59	9.86***
Ni (µg/g)	33.97	34.01	33.33	37.12	35.49	33.51	ns
Cl (µg/g)	40.88	39.19	42.34	47.21	45.27	38.53	ns
Fe (%)	3.98	3.88	4.04	4.11	4.05	3.96	ns
C (%)	1.99	2.39	2.79	3.21	3.66	1.92	0.23***
Ca (%)	0.41	0.41	0.64	0.68	1.01	0.45	0.22***
Mg (%)	0.30	0.31	0.34	0.38	0.39	0.29	0.026***
K (%)	1.03	1.05	1.05	1.08	1.05	1.03	ns
Na (%)	0.217	0.215	0.219	0.226	0.233	0.220	0.005***
Al (%)	6.13	6.10	6.03	5.97	5.67	6.02	0.29*

Table C-9. Results of total elements for soil Standard Reference Materials Till1 and Till3 analysed using XRF and expressed as oxides (%) and (µg/g)

Till1	Measured	Certified	Recovery%	Till3	Measured	Certified	Recovery %
Si O_2 (%)	57.25	60.9	94	Si O2 (%)	66.57	69.1	96
Al_2O_2 (%)	12.43	13.7	91	Al2O2 (%)	10.72	12.2	88
Fe (%)	4.353	4.81	90.5	Fe (%)	2.572	2.78	93
K_2O (%)	1.936	2.22	87	K2O (%)	2.247	2.42	93
Na_2O	2.18	2.71	80	Na2O	2.292	2.64	87
CaO (%)	2.334	2.72	86	CaO (%)	2.377	2.63	90
MgO(%)	1.968	2.15	92	MgO(%)	1.815	1.71	106
S (%)	0.0247	<0.05	<0.05	S (%)	132.1	< 0.05	< 0.05
P (µg/g)	1112	930	119.6	P (µg/g)	569.5	490	116
Mn (µg/g)	1311	1420	92	Mn	451.3	520	87
Zn (µg/g)	89.52	98	91.4	Zn (µg/g)	54.24	56	97
Cu (µg/g)	43.72	47	93	Cu (µg/g)	20.43	22	93
Pb (µg/g)	21.35	22	97.1	Pb (µg/g)	90	26	346
Ni (µg/g)	20.14	24	84	Ni (µg/g)	39.25	39	101
Mo (µg/g)	2.269	2	113.5	MO (µg/g)	1.258	2	63

APPENDIX D

Concentrations of HNO_3/H_2O_2 extractable and ICP-MS determined heavy metals in biosolids amended soils, 2006

Table D-1. Levels of heavy metals extracted using HNO_3/H_2O_2 in composted biosolids amended soils from canola plots in 2006 field experiments, in µg/g

Analytes	Composted biosolids application rates (t/ha)							CRM031-040		
	0	10	30	50	70	Fertilized	$LSD_{0.05}$	Measured	Certified	Recovery
Cu	10	11	13	15	17	2	1.52***	713.9±25.2	805	89
Zn	28	43	44.15	46	64	18	20.83*	947±16	1060	89
Mn	121	128	128	133	133	122	ns	177±4	199	89
Fe	17900	17625	23123	23210	23340	17163	2380***	8925.5±99.7	9810	91
Co	5.2	5.3	5.3	5.4	5.3	5	ns	2.9±0.00	2.96	98
Ni	14.60	15	14.80	15	16	10	ns	17.8±1.3	19.60	91
Pb	7.67	8	9.77	11	14	3.9	2.21**	101.5±0.6	119.00	85
Cr	33.23	31	42.10	42	43	25	5.53**	35.7±0.7	37.20	96
Cd	0.27	0.3	0.17	0.13	0.2	0.20	ns	5.2±0.1	5.74	91
Na	88.6	124	88	81	85	149	ns	471±23	880	53
K	1948	2129	1886	2035	2070	1474	ns	2099±160	2420	87
Mg	471	506	464	550	532	666	ns	2317±21	4290	54
Ca	1335	2427	2012	2555	2508	1436	ns	39350±679	45900	86
Al	34107	34780	32780	33267	32590	34868.30	ns	31845±672	21700	147

Table D-2 Levels of heavy metals extracted using HNO_3/H_2O_2 in dewatered biosolids amended soils from canola plots in 2006 field experiments, in µg/g

Analytes	Dewatered biosolids application rates (t/ha)							CRM031-040		
	0	5	25	45	65	Fertilized	$LSD_{0.05}$	Measured	Certified	Recovery
Cu	1.8	1.4	6	17	16	2.8	2.82***	727±234	805	90
Zn	10	9	21	27	22	13	10.71*	1258±102	1060	118.7
Mn	121	118	132	154	137	117	19*	190±60	199	96
Fe	20955	17009	23595	28059	26150	17757	2784***	9250.5±99.7	9810	94
Co	3.4	3	4	5.25	4.7	5	0.47***	2.7±1.3	2.96	92
Ni	17	20	23	46	43	11	2.5***	17.2±0.1	19.6	88.5
Pb	7	6	7.4	8.8	8.3	4	0.48***	124.6±10.1	119	105
Cr	42	43	56	99.86	93	27	8.24***	39.9±0.2	37.20	107
Cd	0	0.10	0.03	0.03	0.03	0.1	ns	5.05±0.07	5.74	88
Na	94	107	134	258	198	96	ns	928±123	880	105
K	2495	2665	2866	3775	3401	1694	404.6***	2278±39	2420	94
Mg	792	776	884	1004	888	710	130.8*	3946±180	4290	92
Ca	1027	1088	1048	1872	1375	926	189***	53818±3399	45900	117
Al	29748	29287	31398	34933	31522	36025	ns	28300±502	21700	130

Table D-3. Levels of heavy metals extracted using HNO_3/H_2O_2 in dewatered biosolids amended soils from oats plots in 2006 field experiments, in µg/g

Analytes	Dewatered biosolids application rates (t/ha)							CRM031-040		
	0	5	25	45	65	Fertilized	LSD $_{0.05}$	Measured	Certified	Recovery
Cu	10	11	14	19	18	3	2.16***	718.9±18.2	805	89
Zn	13	14	21	27	27	16	4.95***	935±14	1060	88
Mn	125	123	131	134	131	121	7.10*	184.8±2.5	199	93
Fe	17900	17125	22937	23737	23033	17150	1257.4***	9250.5±99.7	9810	94
Co	3	3	4	4	3	5	ns	1.9±0.6	2.96	64
Ni	16	16	17	18	16	11	ns	17.4±0.1	19.60	89
Pb	7	7	9	9	9	4	0.55***	105.9±0.5	119	89
Cr	35	32	44	45	44	24	2.79***	40±0.2	37.20	107
Cd	nd	nd	nd	nd	nd	nd	-	5.1±0.1	5.74	88
Na	69	97	225	145	124	386	ns	664±171	880	75
K	2495	2471	2520	2558	2227	1577	216.8*	2278±39	2420	94
Mg	896	858	882	902	793	690	ns	3020±45	4290	70
Ca	1617	1429	1609	1822	1367	1171	ns	40877±955	45900	89
Al	30590	31055	30947	31990	29857	36425	ns	28655±502	21700	132

Table D- 4. Levels of heavy metals extracted using HNO_3/H_2O_2 in composted biosolids amended soils from oats plots in 2006 field experiments, in µg/g

Analytes	Composted biosolids application rates (t/ha)							CRM031-040		
	0	10	30	50	70	Fertilized	LSD $_{.05}$	Measured	Certified	Recovery
Cu	2.3	4.5	8.1	12.4	19.6	2.4	3.72***	705±31	805	88
Zn	15.7	30.4	45.5	44.0	62.5	10.8	20**	928±25	1060	88
Mn	119.7	116.9	126.9	124.0	132.0	116.4	ns	178.5±9.3	199	90
Fe	16457	17045	22157	22990	22475	17007	714***	8635±74	9810	88
Co	5.0	5.0	5.1	5.4	5.4	6.6	ns	2.9±0.2	2.96	100
Ni	9.2	10	11	12	12.5	10.0	1.45**	12.8±0.4	19.60	65
Pb	4.7	5.9	7.1	8.0	11.0	4.6	2.25**	98±5.80	119	82
Cr	22.6	22.7	33.3	35	35.0	24.4	1.44***	29.6±1.5	37	80
Cd	0.1	0.0	0.0	0.0	0.0	0.1	ns	4.9±0.2	5.74	84
Na	198.7	80.8	151.9	151.7	55.1	128.3	ns	571±154	880	65
K	1326	1477	1614	1822	1880	1396	158***	1598±16	2420	66
Mg	2880	2987	2910	2643	4964	1663	ns	40654±2312	45900	89
Ca	619	677	745	844	984	645	89***	3254±1062	4290	76
Al	33730	35180	35383	36033	34625	34772	ns	33640±1032	21700	155

Table D-5 Results of HNO_3/H_2O_2 extractable heavy metals in CRM031-040 (Sewage sludge standard reference material) analysed using ICP-MS in 2006 experiment (µg/g).

Recoveries of CRM31-040 before analysis of dewatered biosolids amended soil samples taken from canola plots, µg/g			
Elements	Measured	Certified	Recovery (%)
Cu	727 ± 23	805 ± 91.1	90
Zn	1300 ± 100	1060 ± 88.6	119
Mn	190 ± 60	199 ± 24.6	96
Fe	9300 ± 100	9810 ± 824	94
Ni	17.4 ± 0.1	19.60 ± 3.34	89
Pb	125 ± 10	119 ± 17.77	105
Recoveries of CRM31-040 before analysis of dewatered biosolids amended soil samples taken from oats plots, µg/g			
Cu	720 ± 20	805 ± 91.1	89
Zn	930 ± 10	1060 ± 88.6	88
Mn	185 ± 3	199 ± 24.6	93
Fe	9300 ± 100	9810 ± 824	94
Ni	17.4 ± 0.1	19.60 ± 3.34	89
Pb	106 ± 1	119 ± 17.77	90
Recoveries of CRM31-040 before analysis of composted biosolids amended soil samples taken from canola plots, µg/g			
Cu	720 ± 30	805 ± 91.1	89
Zn	950 ± 20	1060 ± 88.6	89
Mn	177 ± 4	199 ± 24.6	89
Fe	8900 ± 100	9810 ± 824	91
Ni	18 ± 1	19.60 ± 3.34	91
Pb	102.0 ± 0.6	119 ± 17.77	85
Recoveries of CRM31-040 before analysis of composted biosolids amended soil samples taken from oats plots, µg/g			
Cu	710 ±30	805 ± 91.1	88
Zn	930 ± 25	1060 ± 88.6	88
Mn	179 ± 9	199 ± 24.6	90
Fe	8640 ± 74	9810 ± 824	88
Ni	12.8 ± 0.4	19.60 ± 3.34	65
Pb	98 ± 6	119 ± 17.77	82

The CRM031-040 (Sewage sludge standard reference material) was analysed using ICP-MS in duplicates and values indicate mean ± sd for each of the elements.

Appendix E

Effect of biosolids applications on soil temperature and seed germination

Table E-1. Effect of dewatered biosolids and composted biosolids applications on canola seed germination percentage

Dewatered biosolids rates (t/ha)	canola seedlings/m^2	canola seedlings/m^2 germinated (%)	Average soil temperature (^{0}C) canola plots	Composted biosolids rates(t/ha)	canola seedlings/m^2	Canola seedlings/m^2 germinated (%)	Average soil temperature (^{0}C) canola plots
0	53	42 (17)	6.16 (0.89)	0	38	30.7 (14.7)	5.60 (0.85)
5	68	54.5 (23.8)	5.99 (1.31)	10	75	60 (18)	7.03 (0.18)
25	90	71.4 (10.4)	7.55 (0.11)	30	96	76.7 (0.92)	6.53 (0.94)
45	109	86.8 (20.1)	8.15 (0.12)	50	110	87.3 (7.94)	8.22 (0.04)
65	115	91.5 (10.5)	6.47 (0.62)	70	104	82.5 (24.8)	8.11 (0.08)
Fertilized	58	46 (11.4)	7.17 (0.20)	Fertilized	56	45 (23.24)	6.25 (1.11)
F-test	0.014	P< 0.014	0.009	F-test	0.004	P<0.004	P<0.001
LSD $_{0.05}$	37.1	29.46*	1.11**	LSD $_{0.05}$	33.2	26.41**	1.03***
R^2	0.99	0.99	-	R^2	0.95	0.95	68.2
CV (%)	32.1	36.8	12.3	CV (%)	35.8	40.2	15.01

The superscripts *, **, and ***refers to significant treatment effects in ANOVA at $p < 0.05$ $p < 0.01$ and $p< 0.001$ respectively. Figures in parenthesis show standard deviations of triplicate measurements

APPENDEX F

Pearson correlation coefficient matrixes between ICP-MS and XRF spectrometry determined heavy metals in dewatered and composted biosolids amended soil samples taken from canola plots, 2006.

Table F-1 Pearson correlation coefficient matrixes between ICP-MS (HNO_3/H_2O_2 extractable) and XRF spectrometry determined heavy metals in dewatered and composted biosolids amended soil from canola plots, 2006

Analyte	Dewatered biosolids amended soil samples taken from canola plots, 2006											
	CuI	CuX	ZnI	ZnX	MnI	MnX	FeI	FeX	NiI	NiX	PbI	PbX
CuI	1											
CuX	0.90**	1										
ZnI	0.69**	0.60*	1									
ZnX	0.94**	0.96**	0.63*	1								
MnI	0.72**	0.69**	0.71**	0.70**	1							
MnX	0.68**	0.82**	0.45ns	0.80**	0.64**	1						
FeI	0.85**	0.76**	0.74**	0.77**	0.77**	0.51*	1					
FeX	0.58*	0.61*	0.60*	0.50ns	0.58*	0.43ns	0.65**	1				
NiI	0.97**	0.91**	0.70**	0.95**	0.76**	0.76**	0.79**	0.54*	1			
NiX	0.47ns	0.59*	0.10ns	0.55*	0.45ns	0.75**	0.34ns	0.25ns	0.52*	1		
PbI	0.91**	0.85**	0.67**	0.84**	0.80**	0.60*	0.94**	0.72**	0.86**	0.40ns	1	
PbX	0.17ns	0.26ns	0.28ns	0.15ns	0.15ns	0.10ns	0.33ns	0.25ns	0.13ns	0.20ns	0.24ns	1
Composted biosolids amended soil samples taken from canola plots, 2006												
CuI	1											
CuX	0.87**	1										
ZnI	0.65**	0.44ns	1									
ZnX	0.90**	0.95**	0.44ns	1								
MnI	0.71**	0.54*	0.24ns	0.67**	1							
MnX	0.66**	0.80**	0.43ns	0.72**	0.19ns	1						
FeI	0.81**	0.78**	0.36ns	0.83**	0.67**	0.61*	1					
FeX	0.79**	0.85**	0.37ns	0.86**	0.45ns	0.73**	0.83**	1				
NiI	0.38ns	0.13ns	0.65**	0.24ns	0.41ns	0.05ns	0.21ns	0.06^{ns}	1			
NiX	-0.3ns	-0.2^{ns}	0.04ns	-0.2ns	-0.2ns	-0.06	-0.57*	-0.4^{ns}	0.04^{ns}	1		
PbI	0.70**	0.70**	0.42ns	0.64**	0.39ns	0.67**	0.54*	0.66**	0.05ns	0.09ns	1	
PbX	0.55*	0.59*	0.03ns	0.69**	0.61*	0.32ns	0.55*	0.48ns	0.22ns	-0.4ns	0.29ns	1

The subscripts I and x denotes ICP-MS and XRF determined heavy metals respectively, whereas, *, ** and ns refers to significance for Pearson correlation coefficients at $p < 0.05$, $p < 0.01$ levels and not significant respectively.

Table F-2 Pearson correlation coefficient matrixes between ICP-MS (HNO_3/H_2O_2 extractable) and XRF spectrometry determined heavy metals in dewatered and composted biosolids amended soil from oats plots, 2006

Analyte	Dewatered biosolids amended soil samples taken from oats plots, 2006											
	CuI	CuX	ZnI	ZnX	MnI	MnX	FeI	FeX	NiI	NiX	PbI	PbX
Cu_I	1											
Cu_X	0.85**	1										
ZnI	0.96**	0.85**	1									
ZnX	0.82**	0.95**	0.83**	1								
MnI	0.83**	0.60*	0.84**	0.60*	1							
MnX	0.65**	0.84**	0.64**	0.81**	0.41ns	1						
FeI	0.89**	0.84**	0.84**	0.80**	0.80**	0.72**	1					
FeX	0.77**	0.89**	0.76**	0.83**	0.66**	0.87**	0.89**	1				
NiI	0.46ns	0.19ns	0.39ns	0.15ns	0.76**	0.13ns	0.49ns	0.36ns	1			
NiX	0.23ns	0.49ns	0.11ns	0.41ns	0.02ns	0.59*	0.28ns	0.53*	0.08ns	1		
PbI	0.90**	0.80**	0.83**	0.74**	0.84**	0.66**	0.98**	0.87**	0.58*	0.31ns	1	
PbX	0.62*	0.81**	0.67**	0.82**	0.50ns	0.77**	0.65**	0.81**	0.19ns	0.42ns	0.59*	1
Composted biosolids amended soil samples taken from oats plots, 2006												
CuI	1											
CuX	0.92**	1										
ZnI	0.82**	0.71**	1									
ZnX	0.91**	0.99**	0.70**	1								
MnI	0.57*	0.46ns	0.73**	0.50ns	1							
MnX	0.13ns	0.09ns	-0.04	0.05ns	-0.20ns	1						
FeI	0.76**	0.69**	0.72**	0.69**	0.51*	0.01ns	1					
FeX	0.54*	0.45ns	0.40ns	0.42ns	0.22ns	0.75**	0.62*	1				
NiI	0.78**	0.77**	0.60*	0.80**	0.50ns	0.01ns	0.79*	0.49ns	1			
NiX	0.16ns	0.06ns	0.01ns	0.04ns	-0.12ns	0.89**	0.11	0.80**	0.16ns	1		
PbI	0.94**	0.85**	0.90**	0.84**	0.71**	0.08ns	0.71	0.49ns	0.75**	0.08ns	1	

The subscripts I and x denotes ICP-MS and XRF determined heavy metals respectively, whereas, *, ** and ns refers to significance for Pearson correlation coefficients at $p < 0.05$, $p < 0.01$ levels and not significant respectively.

APPENDIX G

Concentrations of metals in canola and oats leaves extracted using HNO_3/H_2O_2 analysed by ICP-MS

Table G-1. Effect of various dewatered biosolids application rates on concentrations of metals in canola and oats leaves in 2006 field experiment (in µg/g)

Metals in canola leaves									
Analytes	Dewatered biosolids application rates (t/ha)						SRM 1573a		
	0	5	25	45	65	LSD $_{0.05}$	Measured	Certified	Recovery
Cu	10.27	10.30	10.40	10.67	15.8	8.25*	3.4±1.6	4.7	72
Zn	63.57	60.97	77.90	81.63	115.4	16.36*	23.5±0.4	30.9	76
Mn	34.60	48.05	52.43	45.03	54.07	12.4*	197±7	246	80
Co	0.27	0.20	0.13	0.13	0.60	ns	0.4±0.0	0.57	70
Cd	0.57	0.15	0.50	0.23	0.63	ns	1.2±0.1	1.52	76
K	37550	40700	39637	43273	41493	ns	21560±848	27000	80
Mg	3875	3603	3538	3865	3710	ns	8507±281	12000	71
Ca	16613	14990	16013	17043	17403	ns	40145±1407	50500	80
Metals in oats leaves									
Cu	3.55	4.43	5.53	6.93	7.02	0.77***	5.7±0.9	4.7	121
Zn	16.13	37.10	47.55	56.62	61.37	8.95***	21.5±0.9	30.9	70
Mn	74.92	97.2	67.05	56.13	77.25	12**	181.7±0.6	246	74
Fe	88.58	104.85	80.65	83.90	107.45	ns	323±11	368	88
Co	0.17	0.10	0.22	0.07	0.07	ns	0.45±0.0	0.57	79
Cd	0.15	0.18	0.10	0.08	0.10	ns	0.98±0.04	1.52	64
Ni	0.75	1.10	1.78	1.70	1.68	0.44**	1.18±0.11	1.59	74
Mo	2.03	1.08	1.35	1.33	0.78	0.72*	0.35±0.00	0.46	76
K	29297	39865	41297	44288	52238	7255***	20075±177	27000	74
Ca	2269	2791	2757	2939	3550	650*	37863±272	50500	75
Al	16.75	21.52	31.87	17.85	47.55	19.6*	438±399	598	73

Table G-2. Effect of various composted biosolids application rates on concentrations of metals in canola and oats leaves in 2006 field experiment , (in µg/g)

Metals in canola leaves									
Analytes	Composted biosolids application rates (t/ha)						SRM 1573a		
	0	10	30	50	70	LSD $_{0.05}$	Measured	Certified	Recovery
Cu	8.20	10.50	10.23	9.05	11.95	1.51**	3.05±0.21	4.7	65
Zn	46.4	34.20	51.25	83.37	53.03	26.19*	25±7.5	30.9	81
Mn	39.53	45.80	38.80	36.07	39.30	ns	197±21	246	80
Fe	124	128	138	75.4	135	ns	174±39	368	47
Co	0.17	0.15	0.13	0.30	0.13	ns	0.4±0.0	0.57	70
Cd	0.47	0.45	0.37	0.40	0.23	ns	1.2±0.2	1.52	76
K	44080	45705	45173	47150	45837	ns	21600±2390	27000	80
Mg	4538	3646	4171	4552	3964	ns	8508±1139	12000	71
Ca	22697	22420	21663	21400	20167	ns	40415±4391	50500	80
Metals in oats leaves									
Cu	3.83	4.28	4.65	5.08	5.53	1.04*	3.18±0.74	4.70	68
Zn	18.28	22.33	40.47	42.38	46.28	14.46**	19±6	30.90	62
Mn	57.00	41.03	42.45	31.38	27.53	11.64***	180±0.5	246.00	73
Fe	74.27	74.40	65.50	72.95	70.77	ns	324±1	368.00	88
Co	0.07	0.13	0.05	0.05	0.05	ns	0.45±0.00	0.57	79
Cd	0.05	0.03	0.23	0.20	0.13	0.13*	1.05±0.00	1.52	69
Ni	1.17	1.28	1.57	1.80	1.20	ns	0.9±0.00	1.59	57
Mo	1.20	1.00	0.93	2.48	1.20	ns	0.4±0.00	0.46	87
Pb	0.08	0.02	0.10	0.17	0.23	0.11*	0.25±0.04	nc	nc
K	32787	34980	36835	40230	38010	ns	20425±42	27000	76
Mg	832	763	964	1069	696	ns	8118±88	12000	68
Ca	3213	2710	2875	2357	2526	ns	38602±88	50500	76
Al	23.27	23.70	37.72	56.43	35.30	20.64*	561±205	598	94

Values indicate means of triplicate measurements, whereas, the superscripts *, **, *** and ns refers to significant treatment effect in ANOVA (F-test) at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively. The nc refers to concentrations not certified.

Table G-3. Effect of various dewatered biosolids application rates on concentrations of metals in canola and oats leaves in 2007 field experiment, (in µg/g)

Metals in canola leaves									
Analytes	Dewatered biosolids application rates (t/ha)						SRM 1573a		
	0	5	25	45	65	LSD $_{0.05}$	Measured	Certified	Recovery
Cu	7.78	6.88	6.47	6.67	7.13	ns	3.33±0.04	4.7	69
Zn	102.58	95.30	177.05	69.53	166.57	ns	26±0.00	30.9	84
Mn	31	32	37	60	118	28.9*	197±1	246	80
Fe	338	313	283	287	284	ns	356±1	368	97
Co	0.25	0.38	0.17	0.15	0.23	ns	0.5±0.00	0.57	88
Ni	1.30	1.40	1.45	1.27	1.90	ns	0.95±0.00	1.59	60
Mo	2.30	1.38	0.97	0.60	0.53	0.45***	0.25±0.00	0.46	54
Na	2133	2114	3137	3186	3214	779*	87±6.4	136	64
K	29978	36733	33623	37503	40393	ns	20753±11	27000	77
Mg	5240	4762	5422	5427	4850	ns	8862±11	12000	74
Ca	20697	21002	19940	19010	21130	ns	40017±290	50500	79
Metals in oats leaves									
Cu	5.15	4.88	4.87	5.70	6.88	0.66**	3.1±0.2	4.7	65
Zn	51.1	22.73	45.60	37.03	47.63	ns	36.85±0.0	30.9	119
Mn	32.20	31.88	34.00	39.11	55.09	6.8***	177±1	246	72
Fe	69.50	67.00	65.25	58.58	59.58	ns	214±4	368	58
Cd	0.30	0.08	0.20	0.13	0.28	ns	1.1±0.1	1.52	72
Pb	0.98	1.05	0.60	0.75	0.60	ns	0.27±0.04	0.46	59
K	38803	39327	43977	46212	44439	5510*	19189±184	27000	71
Mg	1517	1450	1636	1649	1697	171*	7832±25	12000	65
Ca	2738	2779	3296	3551	3929	838*	35899±286	50500	71

Table G-4. Effect of various composted biosolids application rates on concentrations of metals in canola and oats tissue in 2007 field experiment (in µg/g)

Metals in canola leaves									
Analytes	Composted biosolids application rates (t/ha)						SRM 1573a		
	0	10	30	50	70	LSD $_{0.05}$	Measured	Certified	Recovery
Cu	6.35	7.38	6.60	5.77	5.63	0.70**	4.08±0.11	4.70	87
Zn	63.42	97.50	61.20	59.63	103.4	27.3*	45±6	30.90	120
Mn	32.77	33.03	42.82	43.88	33.22	ns	201±0.8	246.00	82
Fe	374	253	288	252	287	43**	372±6	368.00	101
Ni	1.97	0.83	0.77	0.70	0.62	0.34***	1.35±0.07	1.59	85
Mo	0.97	0.48	0.75	0.67	1.07	ns	0.275±0.04	0.46	59
Na	1487	2769	3516	5284	5710	767***	99±23	136	84
K	35613	33914	35431	39131	36511	ns	22206±184	27000	82
Mg	4057	5415	4637	6470	4671	1231*	9163±7	12000	76
Ca	23826	18767	22481	18102	23089	4074*	41280±265	50500	82
Metals in oats leaves									
Cu	6.42	5.63	5.17	7.50	5.63	ns	4.1±0.9	4.7	86
Mn	31.23	30.07	23.47	24.00	22.63	5.34*	168±8	246	68
Fe	81.33	62.50	46.67	67.50	54.00	ns	195±18	368	53
Cd	0.88	0.00	0.82	0.07	0.03	ns	1.1±0.1	1.52	72
Ni	1.40	1.80	0.93	0.88	1.02	ns	0.9±0.5	1.59	53
Mo	1.18	0.85	0.75	1.05	0.87	ns	0.25±0.07	0.46	54
K	35776	37244	37123	35214	39889	ns	18296±955	27000	68
Mg	811	1453	1441	1450	1747	171***	7474±308	12000	62
Ca	2479	2762	3522	3075	3236	432**	34336±1439	50500	68
Al	646	776	402	501	432	ns	445±135	598	74

Values indicate means of triplicate measurements, whereas, *, **, *** and ns refers to significant treatment effect in ANOVA (F-test) at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant respectively.

Table G-5 The maximum mass of biosolids that can be applied to a representative site low to high risk soils of the NBRP, based on the total soil metals limits

Risk	Representative site	Metal	Total soil Limits (µg/g)	Conc. of metals in biosolids applied at each site (µg/g)	Mass of biosolids that can be applied (t/ha)
Low	Cecil Plains	Cd	2.3	1.9 - 3.5	280 – 970 (Zn)
		Cu	258	355 - 830	
		Zn	370	495 - 1700	
Moderate	Spalding	Cd	1.4	1.8 - 2.2	675 – 730 (Cu)
		Cu	176	315 - 340	
		Zn	290	435 - 500	
High	Flat Paddock	Cd	0.9	4.6 - 5.4	40 – 90 (Cu)
		Cu	30	420 - 880	
		Zn	71	650 - 870	

The soil properties (pH, organic matter, cation exchange capacity and soil texture) for each of the selected sites for NBRP were used to determine the total soil limits for Cd, Cu and Zn. The ambient background concentration was added to the limits obtained for Cu and Zn from their experimental data.
Heavy metals in parenthesis indicate the metals that restrict the amount of biosolids that can be applied
Adapted from the NBRP, draft position paper, 2007

APPENDIX H

Levels of nitrate-N and sulfur in dewatered biosolids and composted biosolids amended soil in the 2006-2007 field experiments, (µg/g)

Table H-1. The impact of dewatered and composted biosolids applications on the levels of NO_3-N leached at various soil depths biosolids amended soils from canola and oats plots, (in µg/g).

Levels of nitrate-N leached at various soil depths in the highest dewatered biosolids (65t/ha) amended soils from canola and oats plots.						
Soil depth (cm)	2006			2007		
	Control plot	Canola plot	Oats plot	Control plot	Canola plot	Oats plot
20	4.61 (0.42)	44.58 (0.51)	49.65 (2.03)	9.7 (0.1)	63.62 (0.72)	135.55 (0.1)
40	7.4 (0)	32.25 (0.23)	33.41 (0.79)	10.01 (0.21)	45.58 (2.63)	92.33 (0.39)
60	8.57 (0.13)	23.16 (0.23)	17.62 (0.15)	1.33 (0)	17.77 (0.1)	60.1 (0.27)
80	5.82 (0.06)	23.49 (0.34)	14.62 (0.45)	1.66 (0.06)	10.73 (0.1)	47.37 (0.21)
100	6.3 (0.19)	18.65 (0.06)	10.21 (0.06)	1.59 (0.06)	2.83 (0.16)	45.85 (0.22)
Levels of NO_3-N leached at various soil depths in the highest composted biosolids (70t/ha) amended soils from canola and oats plots.						
20	4.61 (0.42)	31.51 (0.35)	84.08 (0.39)	9.7 (0.1)	18.5 (0.27)	0.8 (0.1)
40	7.4 (0)	34.56 (0.12)	58.62 (0.45)	10.01 (0.21)	15.94 (0.22)	0 (0)
60	8.57 (0.13)	20.9 (0.07)	35.8 (0.66)	1.33 (0)	6.09 (0)	0 (0)
80	5.82 (0.06)	5.73 (0.12)	12.14 (0.42)	1.66 (0.06)	3.52 (0.06)	9.09 (0.11)
100	6.3 (0.19)	6.81 (0.12)	13.86 (0.18)	1.59 (0.06)	4.11 (0)	5.97 (0)

Table H-2. Changes in the average concentrations of soil total sulphur concentrations due to dewatered and composted biosolids application rates in 2006 and 2007 field experiment

Total sulphur in dewatered biosolids treated plots in 2006-2007 field experiments				
Composted biosolids rates(t/ha)	Amended soil samples from canola plots		Amended soil samples from oats plots	
	Total S (µg/g) 2006	Total S(µg/g) 2007	Total S (µg/g)) 2006	Total S (µg/g) 2007
0	533	248	532	288
5	573	316	507	353
25	606	485	580	507
45	769	585	754	647
65	836	727	673	713
Fertilized	633	279	551	352
LSD $_{0.05}$	118***	51.85***	136.4**	79***
Total sulfur in composted biosolids treated plots in 2006-2007 field experiments				
0	576	258	665	394
10	564	385	685	345
30	614.3	568	557	430
50	738	791	846	649
70	754	927	999	790
Fertilized	598	321	592	309
LSD $_{0.05}$	74.8***	105.5***	254*	43.84***

Values indicate means of triplicate measurements, whereas, ***, ** and * refers to significant treatment effect in ANOVA (F-test) at p < 0.05, p < 0.01 and p < 0.001 level respectively.

APPENDIX I

Plant nutrient concentration ranges at different growth stages and plant parts of canola and oats

Table I-1 Concentrations of various nutrients in different plant parts at different phonological growth stages of canola crop

Nutrient	Growth stage	Plant part	How established	Deficient	Marginal	Critical deficiency	Adequate	High	Critical toxic	Country	Reference no.
N%	4-5 leaf stage a	WS	Field	na	na	6.3	na	na	na	Australia	399
	5-6 leaf stage a	WS	Field	na	na	5.8-6.00	na	na	na	Australia	399
	Buds visible a	WS	Field	na	na	3.8-4.9	na	na	na	Australia	399
	4-5 leaf stage b	PYML	Field	na	na	4.5	na	na	na	Australia	399
	5-6 leaf stage b	PYML	Field	na	na	4.2-4.5	na	na	na	Australia	399
	Buds visible b	PYML	Field	na	na	2.0-2.9	na	na	na	Australia	399
	4-5 leaf stage a	PYML	Field	na	na	4.6	na	na	na	Australia	399
	5-6 leaf stage a	PYML	Field	na	na	4.2	na	na	na	Australia	399
	Buds visible a	PYML	Field	na	na	1.9-2.9	na	na	na	Australia	399
NO_3 (mg/kg)	4-5 leaf stage b	PYML	Field	na	na	1620	na	na	na	Australia	399
	5-6 leaf stage b	PYML	Field	na	na	1550-1620	na	na	na	Australia	399
	Buds visible b	PYML	Field	na	na	530-480	na	na	na	Australia	399
	4-5 leaf stage b	PYML	Field	na	na	16,500	na	na	na	Australia	399
	5-6 leaf stage b	PYML	Field	na	na	16000-15,500	na	na	na	Australia	399
	Buds visible b	PYML	Field	na	na	4600-4700	na	na	na	Australia	399
	4-5 leaf stage b	PYML	Field	na	na	17,300	na	na	na	Australia	399
	5-6 leaf stage b	PYML	Field	na	na	14,400-15,400	na	na	na	Australia	399
	Buds visible b	PYML	Field	na	na	3600-4700	na	na	na	Australia	399
P%	120DAS c	YMB L4-L5	Field	0.17-0.27	na	0.28-0.32	0.3-0.48	na	na	Australia	743
K%	Pre FI a	YML	Lit.	<1.6	1.8-1.9	na	2.8-5.5	6.5-8.0	na	Australia	1053
Ca%	Pre FI a	YML	Lit.	na	0.8-1.2	na	1.4-3.0	na	na	Australia	1053
Mg%	Pre FI a	YML	Lit.	0.14	0.16-0.19	na	0.21-0.65	na	na	Australia	1053
Na%	Pre FI	YML	Lit.	na	na	na	0.02-0.5	0.7-1.1	na	Australia	1053
Cu (mg/kg)	Pre FI	YML	Lit.	na	2-3	na	4-25		na	Australia	1053
Zn (mg/kg)	Pre FI	YML	Lit.	<12	12-17	na	21-55		na	Australia	1053
Mn (mg/kg)	Pre FI	YML	Lit.	na	na	na	30-250	300-400	530-3650	Australia	1053

Note: a- stands for predictive for 90% maximum seed yield; b-diagnostic for 90% maximum shoot dry weight; c- for maximum vegetative and oil yield
Ws- whole shoot: PYML- petioles of young matured leaf; YMB- young matured blade; YML- young matured leaf; Pre FI- pre flowering stage; Mid-late till- mid to late tillering stage; Lit- literature and na- data not available. Extracted from Plant Analysis: An Interpretation manual, edited by D.J. Reuter, D.J. and Robinson, J.B., 1997

Table I-2 Concentrations of various nutrients in different plant parts at different phonological growth stages of oats crop

Nutrient	Growth stage	Plant part	How established	Deficient	Marginal	Critical deficiency	Adequate	High	Critical toxic	Toxic	Country	Reference no.
N%	Mid-late till	YMB	Field	< 3.4	3.4	na	3.5-5.4	5.5-6.5	na	> 6.5	Australia	1053
P%	Mid-late till	YMB	Field	0.24	0.24-0.29	na	0.3-0.5	0.6-0.7	na	> 0.7	Australia	1053
K%	Mid-late till	YMB	Field	< 1.5	1.5-2.5	na	2.4-4.0	4.1-5.5	na	> 6.0	Australia	1053
Ca%	Mid-late till	YMB	Field	na	< 0.18	na	0.21-0.4	0.6-0.7	na	na	Australia	1053
Mg%	Mid-late till	YMB	Field	< 0.11	0.11-0.12	na	0.13-0.3	na	na	na	Australia	1053
Na%	Mid-late till	YMB	Field	na	na	na	< 0.5	0.6-0.7	na	> 0.8	Australia	1053
Cu (mg/kg)	Mid-late till	YMB	Field	na	2.4	na	5-50	na	na	na	Australia	1053
Zn(mg/kg)	Mid-late till	YMB	Field	< 14	14	na	15-70	na	na	na	Australia	1053
Mn	Mid-late till	YMB	Field	< 12	12-24	na	25-300	400-600	na	na	Australia	1053

Note: YMB: young matured blade, Mid-late till: mid to late tillering stage, Na: data not available
Extracted from Plant Analysis: An Interpretation manual, edited by D.J. Reuter, D.J. and Robinson, J.B., 1997

APPENDIX J

Concentrations of DTPA extractable and ICP-MS determined metals in dewatered biosolids and composted biosolids amended soil

Table J-1 DTPA extractable concentrations of heavy metals in dewatered biosolids amended soils from canola plots in 2006 and 2007 experiments (expressed in mg/kg)

Dewatered biosolids treated canola plots, 2006							
Dewatered biosolids (t/ha)	Cu	Zn	Mn	Fe	Co	Ni	Pb
0	1.2 ± 0.1	3.5 ± 1.5	23 ± 2	66 ± 13	0.4 ± 0.1	1.4 ± 0.1	0.77 ± 0.04
5	1.50 ± 0.04	2.7 ± 0.5	25 ± 3	59 ± 2	0.5 ± 0.1	1.35 ± 0.02	0.79 ± 0.02
25	2.3 ± 0.2	5.3 ± 1.2	23.0 ± 0.2	99 ± 22	0.4 ±0.01	1.5 ± 0.1	0.97 ± 0.12
45	3.5 ± 0.3	10 ± 1	32±1	89 ± 12	0.6 ± 0.01	1.6 ± 0.1	0.93 ± 0.11
65	4.5 ± 0.2	9.5 ± 0.6	32 ± 3	103 ± 8	0.8 ± 0.1	1.63 ± 0.02	1.05 ± 0.04
Fertilized	1.3 ± 0.2	2.4 ± 1.0	26 ± 3	81 ± 35	0.5 ± 0.1	1.4 ± 0.1	0.84 ± 0.12
$LSD_{0.05}$	0.4***	1.96***	3.7***	19.8**	0.14**	0.14**	0.13**
Dewatered biosolids treated canola plots, 2007							
Dewatered biosolids (t/ha)	Cu	Zn	Mn	Fe	Co	Ni	Pb
0	1.80 ± 0.02	1.1 ± 0.1	16 ± 1	46±14	0.24±0.01	1.02±0.04	0.09±0.05
5	1.9 ± 0.2	2.4±0.5	16 ± 1	61±22	0.29±0.05	1±0.1	0.5±0.3
25	4.5±0.8	7.6±1.4	22 ± 1	69±2	0.37±0.01	1.3±0.1	0.6±0.4
45	6.4±0.6	11.0±1.0	25.5±0.4	91±10	0.49±0.04	1.5±0.1	1.4±0.8
65	8.11±0.01	13.9±2.4	27.6±2.7	133±8	0.54±0.06	1.5±0.1	0.6±0.2
Fertilized	1.4±0.2	1.2±0.3	22.5±5.0	43±6	0.5±0.2	1.1±0.1	0.4±0.2
$LSD_{0.05}$	0.79***	2.85***	2.48***	23.8***	0.08***	0.19***	0.80*

The superscripts *, **, ***, and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$ $p \leq 0.001$ and not significant, respectively. Values indicate means ± sd of triplicate measurements

Table J-2 DTPA extractable levels of heavy metals in dewatered biosolids amended soils from oats plots in 2006 and 2007 experiments (mg/kg)

Dewatered biosolids treated oats plots, 2006							
Dewatered biosolids	Cu	Zn	Mn	Fe	Co	Ni	Pb
0	1.4 ± 0.2	1.5 ±0.1	28 ± 2	67 ± 11	0.5 ± 0.1	1.2 ± 0.1	0.7±0.1
5	1.6 ±0 .1	2.3 ±0.5	23 ± 1	69 ± 20	0.5 ± 0.1	1.3 ± 0.1	1.0±0.2
25	2.7 ± 0.4	5.3 ±2.0	27.5± 3.2	71 ± 5	0.5 ± 0.1	1.3 ± 0.1	0.79 ±0.02
45	3.9 ± 0.4	7.5 ±1.5	30.4 ± 2.6	85 ± 14	0.6 ± 0.1	1.3 ± 0.1	0.9±0.1
65	4.5 ± 0.6	9.1 ±1.8	31.0 ± 3.0	145 ± 25	0.6 ± 0.1	1.45 ± 0.02	1.1±0.1
Fertilized	1.4 ± 0.1	2.3 ±0.8	29.5 ± 3.0	65 ± 12	0.6 ± 0.1	1.7 ± 0.1	0.7±0.1
$LSD_{0.05}$	0.8***	2.8***	4.4*	33.6**	ns	0.15*	0.21*
Dewatered biosolids treated oats plots, 2007							
Dewatered biosolids	Cu	Zn	Mn	Fe	Co	Ni	Pb
0	1.54±0.04	0.95±0.7	19.2±2.4	79±22	0.36±0.04	1.23±0.07	0.7±0.1
5	2.8±1.5	2.7±0.6	19.3±1.2	72±2	0.35±0.03	1.23±0.08	0.71±0.02
25	4.4±1.1	7.5±1.7	22.4±2.8	116±22	0.41±0.06	1.42±0.06	0.95±0.06
45	7.9±0.4	14.3±1.2	30.4±2.6	134±16	0.61±0.08	1.74±0.10	0.98±0.09
65	9.7±0.2	16.5±1.7	31.3±0.6	182±28	0.64±0.04	1.79±0.08	1.09±0.04
Fertilized	1.1±0.2	0.6±0.2	20.5±0.6	94±42	0.41±0.05	1.22±0.10	0.69±0.14
$LSD_{0.05}$	1.18***	2.27***	2.97***	39.81**	0.082***	0.13***	0.12***

The superscripts *, **, ***, and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$ $p \leq 0.001$ and not significant, respectively. Values indicate means ± sd of triplicate measurements

Table J-3 DTPA extractable levels of heavy metals in composted biosolids amended soils from canola plots in 2006 and 2007experiments (mg/kg)

Composted biosolids treated canola plots, 2006							
Composted biosolids (t/ha)	Cu	Zn	Mn	Fe	Co	Ni	Pb
0	1.1 ± 0.1	1.2 ± 0.3	24 ± 6	46 ± 16	0.5 ± 0.1	1.0 ± 0.2	0.7 ± 0.2
10	1.3 ± 0.2	2.2 ± 0.2	25 ± 3	45 ± 8	0.4 ± 0.1	1.1 ± 0.2	0.8 ± 0.2
30	1.5 ± 0.2	5.7 ± 1.2	24 ± 3	66 ± 2	0.4 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
50	2.1 ± 0.3	8 ± 2	24 ± 3	69 ± 4	0.5 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
70	2.3 ± 0.2	9.9 ± 1.6	25 ± 1	123 ± 22	0.4 ± 0.01	1.2 ± 0.2	1.1 ± 0.1
Fertilized	1.2 ± 0.1	1.7 ± 0.1	24 ± 2	85 ± 49	0.5 ± 0.1	1.1 ± 0.1	0.8 ± 0.2
LSD $_{0.05}$	0.4***	2.3***	ns	21***	ns	ns	0.25*
Composted biosolids treated canola plots, 2007							
Composted biosolids (t/ha)	Cu	Zn	Mn	Fe	Co	Ni	Pb
0	1.4±0.2	2.0±1.0	14±2	46±12	0.23±0.02	0.9±0.2	0.9±0.4
10	1.5±0.3	4.0±2.0	16±2	22.1±0.1	0.28±0.04	0.9±0.3	0.5±0.3
30	2.1±0.1	8.8±1.4	16±2	62±16	0.26±0.02	0.9±0.1	1.2±0.3
50	2.6±0.2	14.7±0.2	19±1	77±2	0.32±0.03	1.1±0.01	2.04±0.02
70	3.6±0.5	20.0±4.0	19±4	76±2	0.33±0.02	1±0.2	2.2±0.1
Fertilized	1.1±0.1	1.6±0.7	20±7	42±17	0.37±0.24	0.9±0.1	0.60±0.06
LSD $_{0.05}$	0.46***	4.10***	ns	18.9***	0.046**	ns	0.51***
Measured (µg/L)	99	97	99	9942	99	99	94
Certified (µg/L)	99	99	99	9900	99	99	99
Recovery %	100	98	100	101	101	101	95

The superscripts *, **, ***, and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$ $p < 0.001$ and not significant, respectively.

Table J-4 DTPA Extractable levels of heavy metals in composted biosolids amended soils from oats plots in 2006 and 2007 experiments (mg/kg)

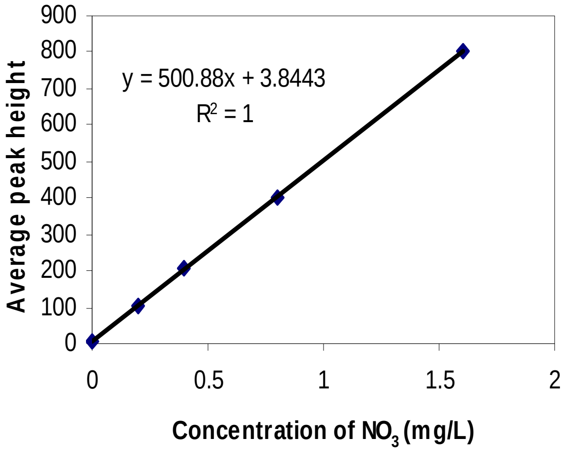
Composted biosolids treated oats plots, 2006							
composted biosolids	Cu	Zn	Mn	Fe	Co	Ni	Pb
0	0.9 ± 0.1	0.7 ±0.5	23.0 ± 3.0	40 ± 6	0.4 ±0.1	0.9 ± 0.2	0.7 ± 0.2
10	1.2 ± 0.2	3.0 ±1.0	22.7 ± 0.4	55 ± 15	0.4 ± 0.1	0.9 ± 0.2	1.0 ± 0.1
30	1.6 ± 0.3	5.5 ± 0.9	22.8 ± 0.2	90 ± 20	0.40 ± 0.04	0.9 ± 0.1	1.0 ± 0.1
50	2.2 ± 0.1	12 ± 1	25.0 ± 1.0	95 ± 15	0.48 ± 0.04	1.1 ± 0.1	1.2 ± 0.2
70	3.4 ± 0.1	18.7 ± 1	25.0 ±4 .0	102 ± 24	0.5 ± 0.1	1.1 ± 0.2	1.5 ± 0.2
Fertilized	1.2 ± 0.3	1.5 ± 0.3	25.5 ± 2.4	77 ± 11	1.4 ± 0.8	1.2 ± 0.3	1.7 ± 0.1
LSD $_{0.05}$	0.32***	1.5***	ns	28.6**	0.1*	ns	0.3**
Composted biosolids treated oats plots, 2007							
composted biosolids (t/ha)	**Cu**	**Zn**	**Mn**	**Fe**	**Co**	**Ni**	**Pb**
0	1.4±0.2	0.50±0.03	17±2	51±16	0.28±0.04	1.21±0.12	0.6±0.1
10	1.8±0.3	3.70±0.01	18±1	60±6	0.29±0.01	1.10±0.06	0.8±0.1
30	2.2±0.2	8.9±1.4	16±2	83±6	0.25±0.02	1.05±0.09	1.0±0.1
50	2.8±0.2	17.7±0.4	17±1	101±8	0.29±0.01	1.14±0.10	1.3±0.1
70	3.4±0.1	23.7±1.4	23±6	154±8	0.36±0.04	1.14±0.05	1.9±0.2
Fertilized	1.3±0.1	0.4±0.1	17±2	81±21	0.28±0.05	1.02±0.19	0.6±0.2
LSD $_{0.05}$	0.4***	1.48***	ns	37.61**	ns	ns	0.26***

The superscripts *, **, ***, and ns refers to significant treatment effects in ANOVA (F-test) at $p < 0.05$, $p < 0.01$ $p \leq 0.001$ and not significant, respectively.

APPENDIX K

Calibration standards used for the determination of NO_3-N and PO_4-P in soil and plant extracts

Concentration (NO_3)	Peak height
0	5
0.2	101.66
0.4	205.33
0.8	404.66
1.6	805.2



Concentration (NO_3)	Peak Height
0	5
0.2	112
0.4	220
0.8	428.66
1.6	838.66

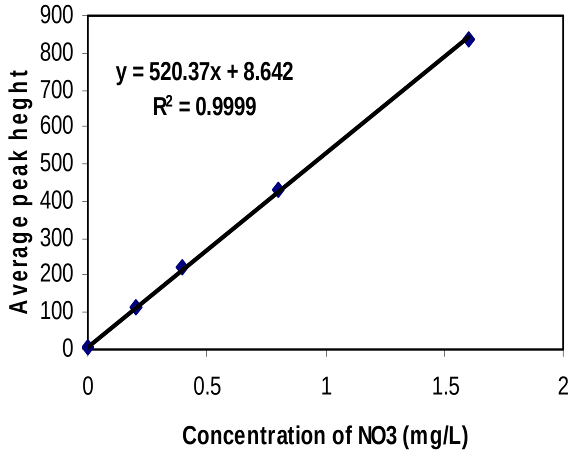


Figure K-1 Shows the calibration curves used to determine the concentrations of NO3-N in soil and plant extracts using flow injection analysis

Concentrations (PO_4)	Peak height
0	1
0.2	47.66
0.4	97
0.6	143
0.8	194.33
1	240.33
1.5	360.66
2	476.33

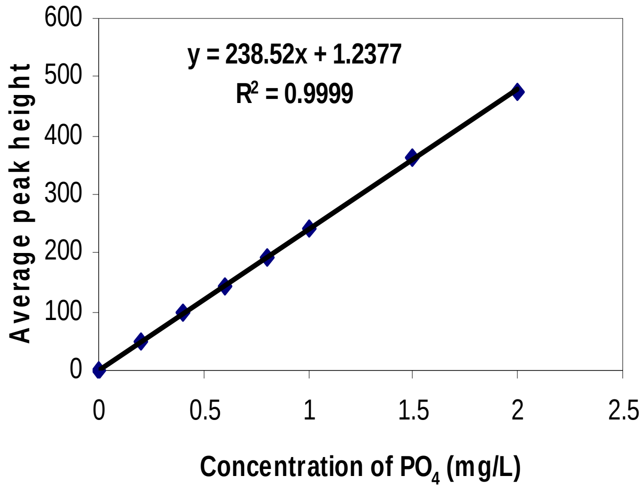


Figure K-2 Shows the calibration curve used to determine the concentrations of phosphorus in soil and in plant extracts using flow injection analysis